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The relationship between lifestyle factors and neurodegeneration in midlife as expressed on arterial spin labelling and structural magnetic resonance imaging

Hinesh Topiwala

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Thesis declaration

I declare that this thesis has been composed solely by myself and that it has not been submitted, in whole or in part, in any previous application for a degree. Except where states otherwise by reference or acknowledgment, the work presented is entirely my own.

Hinesh Topiwala

1st June 2019

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Contents

i. Table of abbreviations

Abbreviation	Explanation
ACC	Anterior Cingulate Cortex
ACE III	Addenbrooke's Cognitive Examination 3rd revision
ADAS-cog	Alzheimer's Disease Assessment Scale – cognitive subscale
ALDS	Academic Medical Center Linear Disability Score
ALFF	Amplitude Low Frequency Fluctuate
AMNART	American National Adult Reading Test
ANOVA	Analysis of the Variance
APOE	Apolipoprotein E
ARIC	Atherosclerosis Risk in Communities
ASL	Arterial Spin Labelling
AUD	Alcohol Use Disorder
BDNF	Brain Derived Neurotrophic Factor
BISQ	Brain Injury Screening Questionnaire
BMI	Body Mass Index
BOLD	Blood Oxygen Dependent
CaPs	Caerphilly Prospective Study
CBF	Cerebral Blood Flow
CCG	Clinical Commissioning Groups
CES-D	Centre for Epidemiologic Studies Depression Scale
CI	Confidence Interval

Abbreviation	Explanation
CMRO2	Cerebral Oxygen Metabolism
CSF	Cerebrospinal Fluid
CVD	Cardiovascular Disease
dACC	dorsal Anterior Cingulate Cortex
DBP	Diastolic Blood Pressure
DMN	Default Mode Network
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, 4th Edition
DSM-V	Diagnostic and Statistical Manual of Mental Disorders, 5th Edition
DWI	Diffusion Weighted Imaging
DWMH	Deep White Matter Hyperintensities
EPAD	European Prevention of Alzheimer's Dementia
EPHPP	Effective Public Health Practice Project Quality Assessment
FDA	Food and Drug Administration
FFQ	Food Frequency Questionnaire
FINGER	Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability
FLAIR	Fluid Attenuation Inversion Recovery
fMRI	functional Magnetic Resonance Imaging
FOAD	Foetal Origins of Adult Disease
GCP	Good Clinical Practice
GMV	Grey Matter Volume
HC	Healthy Controls

Abbreviation	Explanation
HR	Hazard Ratio
IADL	Lawton Instrumental Activities of Daily Life
ICD-10	International Statistical Classification of Diseases and Related Health Problems, 10h Revision
ICV	Intracranial Volume
ICH	International Council on Harmonisation
IGD	Internet Gaming Disorder
MANOVA	Multivariate Analyses of Variance
MAPT	Multidomain Alzheimer Preventive Trial
MRS	Magnetic Resonance Spectroscopy
NA	Not Available
NART	NFL
NINCDS-ARDRA	National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association
NMDA	N-methyl-D-aspartate
NTB	Neuropsychological Test Battery
LCS	Longitudinal Cohort Study
LEQ	Lifetime of Experiences Questionnaire
OR	Odds Ratio
PCC	Posterior Cingulate Cortex
PSS	Post Signal Slowing
PSSI	Power Spectrum Scale Invariance
PSQI	Pittsburgh Sleep Quality Index

Abbreviation	Explanation
PVH	Periventricular Hyperintensities
RAPA	Rapid Assessment of Physical Activity
ReHo	Regional Homogeneity
ROI	Regions Of Interest
RR	Relative Risk
rsFC	resting-state Functional Connectivity
SBP	Systolic Blood Pressure
SD	Standard Deviation
SE	Standard Error
sMRI	structural Magnetic Resonance Imaging
SWI	Susceptibility-Weighted Imaging
WHO	World Health Organisation
WMV	White Matter Volume
WRAP	Wisconsin Registry for Alzheimer's Prevention

ii. Abstract

Introduction: Studies have demonstrated a relationship between neurodegeneration and lifestyle factors. Neurodegeneration in midlife (40 – 59 years old) can be assessed using neuroimaging. The aim of the thesis was to evaluate the relationship between lifestyle factors and neurodegeneration in midlife as expressed on arterial spin labelling and structural magnetic resonance imaging.

Methods: A systematic review of the relationship between lifestyle and neurodegeneration in midlife as expressed on functional magnetic resonance imaging (fMRI) was undertaken. Additionally, the impact of lifestyle on the arterial spin labelling (ASL) and structural magnetic resonance imaging (sMRI) expression of neurodegeneration in a midlife cohort was analytically assessed.

Results: Seven lifestyle factors associated with neurodegeneration in midlife as expressed on fMRI were identified by the systematic review. Using data from the PREVENT Dementia cohort, linear regression analysis demonstrated multiple associations between lifestyle and neurodegeneration in midlife as expressed on ASL and sMRI.

Discussion: The findings from this thesis can guide future analysis of PREVENT Dementia cohort data. Furthermore, the findings from this thesis could be shared with the public through the NHS Health Check in England and the Keep Well Programme in Scotland, to help promote lifestyle interventions to optimise brain health in midlife.

iii. Lay summary

This thesis focused on the effect of lifestyle factors on brain health in midlife as measured on brain scans. Lifestyle describes regular patterns of behaviour which people engage in and midlife has been defined as between the ages of 40 and 59 years. In the first part of the thesis, a medical literature review was undertaken. All published studies on the relationship between lifestyle and brain health in midlife (as measured on brain scans) were systemically reviewed and summarised. The medical literature review identified seven lifestyle factors; alcohol, cognitive training, excessive internet use, fasting, physical training, smoking and substance misuse associated with brain health in midlife. In the second part of the thesis, data from a group of 210 volunteers were analysed. The volunteers underwent brain scans and completed questionnaires about their health and lifestyle. The analyses showed associations between four lifestyle factors (substance misuse, smoking, alcohol and physical training) and brain health, as measured on brain scans. In summary, this thesis has shown a relationship between lifestyle and brain health, as measured on brain scans in midlife. The findings of this thesis should be shared with the public to help them better understand how lifestyle and brain health are interconnected. Looking to future, this thesis, forms an exciting platform for research in the field of lifestyle and brain health to build upon.

1. Introduction

Chapter abstract

Adopting a life course approach to the study of neurodegeneration, highlights a strong body of evidence suggesting an association between lifestyle and neurodegeneration in adulthood. Adulthood encompasses both midlife (40 -59 years old) and late life (60+ years old). Clinical trials have only recently begun to explore the relationship between lifestyle and neurodegeneration. The trials (preDIVA, MAPT and FINGER) have focused on late life risk factor modification and were either unsuccessful or of limited benefit. A logical next step in researching the relationship between lifestyle and neurodegeneration is to focus on an earlier life stage, namely midlife. In midlife, there is increasing evidence that early neurodegenerative changes associated with late life dementia can be assessed using neuroimaging. Therefore, the focus of my thesis will be the relationship between lifestyle factors and neurodegeneration in midlife as expressed on ASL and sMRI. In the study of lifestyle, there is much variation in medical literature about how lifestyle is defined. My thesis will assess lifestyle factors that are in accordance with the World Health Organisation (WHO) definition of lifestyle.

1.1 The importance of adopting a life course approach in the study of neurodegeneration

A life course approach considers factors that act during development and ageing, which might influence disease onset. When a life course approach is applied to the study of neurodegeneration, it may help identify if the accumulation of different types of risk factors such as maternal nutrition, childhood education and adulthood smoking, result in neurodegeneration. Furthermore, a life course approach can help identify if there is a critical period during which an exposure, such as obesity can have adverse or protective effects on neurodegeneration. A life course approach can also help identify sensitive periods. These are time periods when an exposure, such as diet, has a stronger effect on development and subsequent disease risk than it would at other times (1).

Muller et al. (2) outlined a hypothetical model of risk factors for brain ageing (Figure 1) that demonstrates a life course approach. In this model, risk factors were broadly divided into different life stages- the prenatal period, childhood and adolescence, adulthood and old age. Using this model as a framework, the evidence for the presence of risk factors for neurodegeneration in the prenatal period, childhood and adolescence, adulthood and old age have been described and evaluated below. Based on the strength of current evidence, the relationship between lifestyle factors and neurodegeneration has been identified as the focus of this thesis.

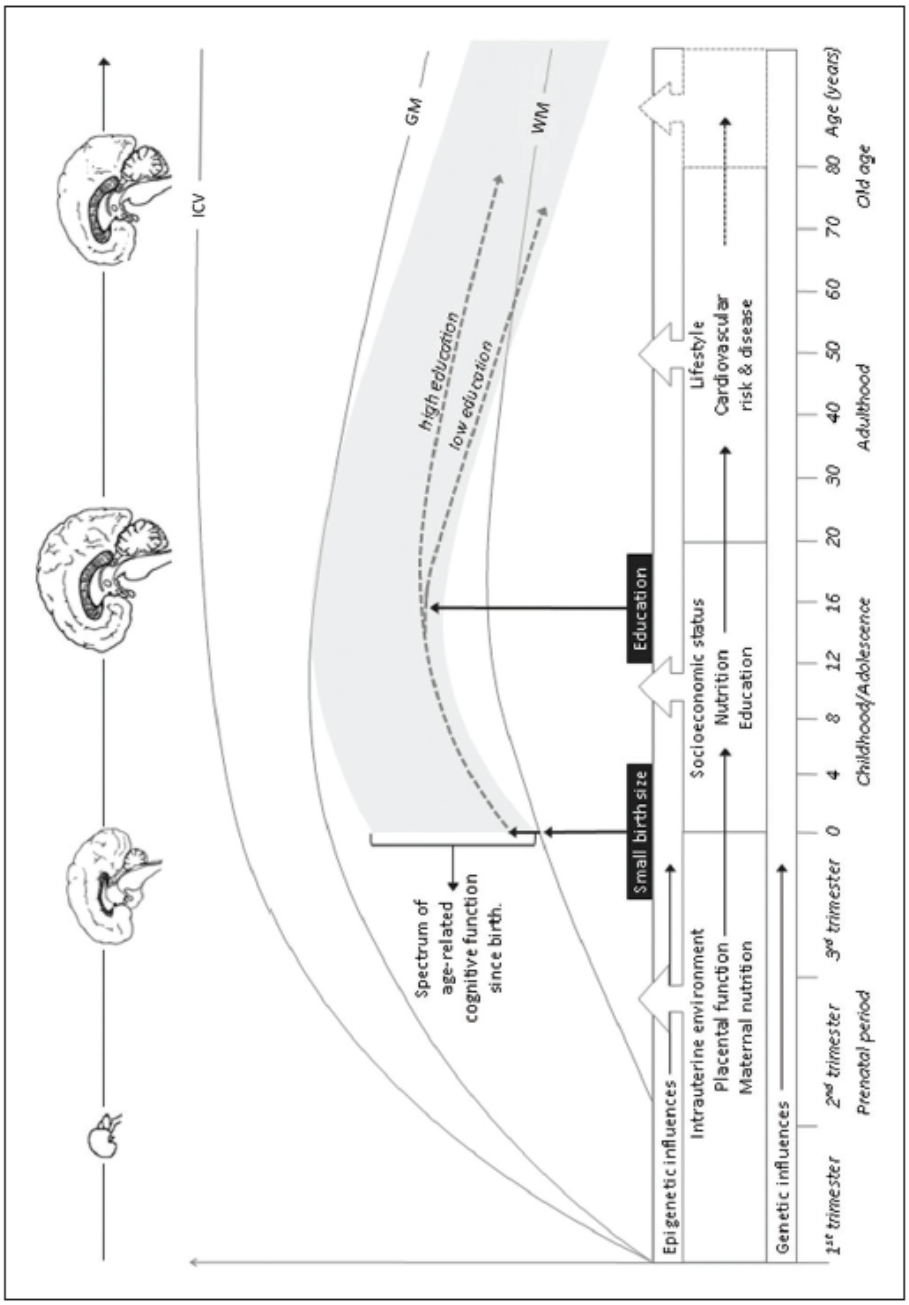

Figure 1: Hypothetical model of the life course of brain ageing (2)

Prenatal period: foetal origins of adult disease and dementia

The foetal origins of adult disease (FOAD) hypothesis proposes causal associations between a disadvantageous early foetal environment, indicated by low birthweight for date and later ischaemic heart disease, hypertension, obesity, and insulin resistance (3). The FOAD hypothesis could be applied to dementia through three possible pathways; firstly, directly effecting the brain during embryogenesis making dementia more likely; secondly, indirectly effecting the brain through a pathway that leads to adult cardiorespiratory disease and hyperglycaemia, and then to dementia in old age; or thirdly, increasing rates of ageing, causing age-dependent disorders to arise prematurely (1). However, it is worth reiterating that the FOAD pathways are hypothetical and yet to be proven. Long term follow-up studies are needed to see if the FOAD hypothesis could be applied to the study of neurodegeneration.

Infancy: nutrition and neurodegeneration

In early life, long-chain polyunsaturated fatty acids are required in large amounts for the rapid brain growth that occurs in the postnatal period (1). In the Helsinki Study of Very Low Birth Weight Adults (birthweight <1500g) there was an association between nutrition and adult neurocognitive outcomes at age 25 years old. A 10 kcal/kg/day higher total energy intake at 3 to 6 weeks of age was associated with 0.21 standard deviation (SD) higher adult IQ (95% confidence interval (CI) 0.07–0.35). Higher carbohydrate and fat intake at 3 to 6 weeks, and higher energy intake from human milk at 3 to 6 and at 6 to 9 weeks were also associated with higher adult IQ. (4). However,

there is a lack of long-term cohort studies linking childhood nutrition to neurodegeneration.

Childhood: socioeconomic status and neurodegeneration

In a longitudinal study of socioeconomic status and cognitive function in 463 individuals in the Seychelles, Kobrosly et al. demonstrated an associated between early life socioeconomic position and cognitive function at 17 years old, including the cognitive domains of calculation (P= 0.02) and language comprehension (P= <0.01) (5). In a case-control study of 239 patients with Alzheimer's dementia, Moceri et al. (6) investigated the risk of Alzheimer's dementia associated with father's occupation, parental age, household size, sibship size and birth order. The study demonstrated that subjects whose fathers were unskilled manual workers or labourers were at higher risk for Alzheimer's dementia (odds ratio (OR) 1.80, 95% confidence interval (CI) 1.19 – 2.73). The investigators in the study postulated that father's occupation reflects not only the level of family income but also the quality of nutrition and housing, and the family's access to medical care. However, the Religious Orders Study, a prospective study of 859 elderly Catholic clergy, found no association between childhood social circumstances and subsequent Alzheimer's dementia (7).

In a review of the early life epidemiology of Alzheimer's dementia, Seifan et al. (8) proposed that although multiple studies agree that a lower early life socioeconomic status is associated with reduced late life cognitive ability, evidence for an association with late life dementia is

equivocal. Further research is needed to clarify the relationship between socioeconomic status and dementia.

Adolescence: education and neurodegeneration

In a recent Lancet commission report on dementia, Livingston et al. (9) calculated that low education is associated with dementia (Relative Risk (RR) 1·59, 95% CI 1·26 – 2·01). In the report, low education, was defined as no secondary school education. However, the evidence on the relationship between dementia and low education is inconsistent. Sharp et al. (10), conducted a systematic review of the relationship between education and dementia. The inclusion criteria were a measure of education, this included years of education and level of educational attainment and a dementia diagnosis by a standardised diagnostic procedure. A total of 88 study populations from 71 articles met the inclusion criteria. Overall, 51 (58%) reported significant effects of low education on risk for dementia, whereas 37 (42%) reported no significant relationship (10).

Mortimer (11) suggested that years of formal education may raise an individual's level of cognitive reserve and thus exert a protective effect against developing dementia. Low education is thought to result in vulnerability to cognitive decline because it results in less cognitive reserve. It has been hypothesised that people with higher levels of education may develop a greater complexity and efficiency of neural networks, meaning as dementia pathology develops, they can actively compensate by drawing on a greater reserve of cognitive processing approaches (12).

A review paper found 22 papers reporting cohort studies of the effects of education, occupation, premorbid IQ and mental activities in incident dementia published up to 2004. The great majority of the studies demonstrated a significant protective effect of these lifetime exposures. The authors summarized all of the studies to calculate the protective effect of higher cognitive reserve and found that it decreased the risk of developing dementia by 46% (13).

Adulthood and old age: cardiovascular risk

Most dementia research largely overlooks vascular disease as a cause. This is thought to reflect the fact that many clinicians and researchers working on dementia, stroke, physical, or psychiatric manifestations are still too often segregated (14). However, there is now decades of data, including work from the Honolulu Asia Aging Study, the Rotterdam Study and the Religious Orders Study that highlights the significance of vascular contributions to dementia, including Alzheimer's dementia (15-17). Over the past 50 years the control of vascular risk factors including diabetes and hypertension, has led to a major decline in the annual risk of stroke. Whether improved control of vascular risk factors has translated to decreased dementia risk is not known but has been suggested(18).

In a meta-analysis of longitudinal studies looking at diabetes as a risk factor for dementia, Cheng et al. showed that subjects with diabetes have a higher risk for Alzheimer's dementia (RR 1.46, 95% CI 1.20-1.77), Vascular dementia (RR 2.48, 95% CI 2.08 - 2.96), any dementia (RR 1.51, 95% CI 1.31 - 1.74) and Mild Cognitive Impairment (RR 1.21, 95% CI 1.02 - 1.45)

than those without diabetes (19). However, in the review, there is no clear distinction between developing diabetes in midlife and late life. Furthermore, for many studies, diabetes was diagnosed using medical records, medication prescribed for diabetes or self-reported. This would miss many individuals with undiagnosed diabetes, who would have been correctly identified in those studies that had used an oral glucose tolerance tests in addition to the measures used to identify diagnosed diabetes.

In a review of modifiable risk factors for Alzheimer's dementia, Barnes et al. (20) observed that midlife obesity is associated with Alzheimer's dementia (RR 1.60, 95% CI 1.34-1.92). This is supported by the findings of a systematic review and meta-analysis of longitudinal cohort studies of the risk of obesity in midlife and late-life in the development of dementia by Pedditizi et al. (21). Inclusion criteria included epidemiological longitudinal studies, published up to September 2014, in participants without cognitive impairment and aged 30 or over at baseline assessment with at least 2 years of follow-up. Of the 1,612 abstracts identified and reviewed, 21 met the inclusion criteria. Review of the 21 papers showed that being obese and below the age of 65 years, had a positive association with dementia (RR 1.41, 95% CI 1.20 – 1.66), but the opposite was seen in those aged 65 and over, (RR 0.83, 95% CI 0.74 – 0.94). This study exemplifies the importance of adopting a life course approach to the study of neurodegeneration (21). There is a biologically plausible association between obesity and dementia. Adiposity may have direct adverse effects on brain tissue through production of

inflammatory cytokines, advanced glycosylation end products and hyperinsulinaemia (22).

Studies of the association of hypertension with the incidence of dementia, further support the necessity of adopting a life course approach. In a literature review, looking at the relationship between hypertension and risk of dementia in late life, Qui et al. identified seven cross-sectional studies that focused on the relationship between blood pressure and dementia in late-life. Of these seven studies, two studies reported an association between low blood pressure and high prevalence of dementia and Alzheimer's dementia. Three studies identified an association of self-reported or clinically diagnosed hypertension with a lower prevalence of dementia and two studies found no association between blood pressure and prevalent dementia and Alzheimer's dementia (23).

In midlife, the association between hypertension and an increased risk of dementia is more robust than in late-life. In four out of five studies identified in a literature review by Qui et al, an association between raised midlife blood pressure and increased risk of late-life dementia and Alzheimer's dementia was evident (23). Furthermore, using data from the Honolulu Heart Program, Stewart et al. (24) have demonstrated evidence supporting an association between midlife hypertension and an increased risk of dementia. A cohort of 3703 Japanese–American men were followed up and the risk for dementia associated with categories of systolic and diastolic blood pressure were assessed. The risk for dementia was greater for groups with a Diastolic Blood Pressure (DBP) of 90–94 mmHg (OR 3.8,

95% CI 1.6–8.7), and DBP of 95 mmHg and over (OR 4.3, 95% CI 1.7–10.8), compared to a DBP of 80 to 89 mmHg. The risk for dementia was greater for a Systolic Blood Pressure (SBP) of 160 mmHg and higher compared to a SBP of 110 to 139 mmHg, (OR 4.8, 95% CI 2.0–11.0) (24).

Adulthood and old age: lifestyle and neurodegeneration

There is a strong body of evidence that lifestyle factors are associated with neurodegeneration. Smoking is associated with multiple health risks and is the chief preventable cause of death worldwide (25). The annual global number of deaths due to tobacco is six million and projected to rise to eight million by 2030 (26). More specifically, there is a robust evidence base for an association between smoking and Alzheimer's dementia. In a systematic review and meta-analysis of modifiable factors associated with cognition and dementia by Beydoun et al., MEDLINE was searched for published literature from January 1990 through to October 2012. In total, 247 studies were retrieved for systematic review and 31 included in the meta-analysis. The study showed an association between being a smoker and Alzheimer's dementia (RR 1.37, 95% CI 1.23-1.52) (27). This finding is supported by transgenic Alzheimer's dementia animal model studies that suggest cigarette smoke increases amyloidogenesis, neuroinflammation and tau phosphorylation (28).

In terms of dose response amongst smokers, the evidence is inconsistent. In the Honolulu Asia Aging Study, amongst current and ex-smokers, the risk for dementia increased with increasing pack-years of exposure up to 'heavy' smoking levels (>40.5 to 55.5 pack-years), but the

risk in ‘very heavy smokers’ (> 55.5 pack-years) was similar to that in ‘light’ smokers (>0 to <26.7 pack-years) (29). Furthermore, in the Chicago Health and Aging Project cohort study there was no association between smoking pack-years with Alzheimer’s dementia amongst current smokers (p=0.88), but amongst ex-smokers there was a significant trend towards a lower risk of incident Alzheimer’s dementia with increasing pack-years of exposure (p=0.02); effect sizes were not reported (30).

There are a large number of observational studies that show an association between physical activity and dementia. Sofi et al. (31) conducted a systematic review of epidemiological studies with a prospective design that assessed the association between physical activity and cognitive decline in people without dementia or cognitive impairment at baseline. There were 15 studies that met the inclusion criteria and the relative risks from the meta-analysis showed a lower risk of cognitive decline for those in the high (RR 0.62, 95% CI 0.54-0.70), and moderate (RR 0.65, 95% CI 0.57-0.75) physical activity level groups compared to those in the low-level group. In a systematic review of the prospective evidence for physical activity and risk of neurodegenerative disease, Hamer et al. (32), searched Medline, the Cochrane Database of Systematic Reviews and Web of Science databases from 1990 to 2007 for prospective epidemiological studies of physical activity and incident dementia, Alzheimer's and Parkinson's disease. There were 16 prospective studies included in the overall analysis. These studies incorporated 163,797 participants without dementia at baseline with 3,219 cases at follow-up. The highest physical activity group compared to the

lowest physical activity group had a smaller risk for dementia (RR 0.72, 95% CI 0.60–0.86) and Alzheimer's dementia (RR 0.55, 95% CI 0.36–0.84). The results suggest that physical activity is inversely associated with risk of dementia and Alzheimer's dementia.

There is a body of evidence showing an association between midlife physical activity and subsequent dementia risk. Rovio et al. (33), assessed the association between leisure time physical activity in midlife and the subsequent development of dementia. Using regression analysis, Rovio et al. (33) demonstrated that leisure-time physical activity in midlife was associated with a decreased risk of dementia in later in life. From a population based cohort of 1449 persons, aged 65–79 years, 117 persons had dementia. Even after adjustments for age, sex, education, follow-up time, locomotor disorders, *APOE* genotype, vascular disorders, smoking, and alcohol drinking, leisure-time physical activity in midlife at least twice a week was associated with a reduced risk of dementia (OR 0·48, 95% CI 0·25–0·91) . Using evidence from a population based study of Swedish twins, Andel et al. (34) demonstrated that exercise in midlife may reduce the odds of dementia in late life. In the case-control analysis, light exercise such as gardening or walking were associated with a reduced odds of dementia compared to hardly any exercise (OR 0.63, 95% CI 0.43-0.91). Additionally, regular exercise involving sports were associated with a reduced odds of dementia compared to hardly any exercise (OR 0.34, 95% CI 0.16-0.72).

Alcohol is implicated as a causal factor for more than 200 diseases and injuries, including major non-communicable diseases such as liver

cirrhosis, some cancers and cardiovascular disease (35). The neurodegenerative effects of alcohol on the brain are well established. It produces cerebral volume loss, including white matter changes related to memory processing and visuospatial functioning (36). Additionally, neuronal loss in alcohol dependent patients has also been described in the frontal association cortex, hypothalamus, cerebellum, hippocampus, amygdala and locus coeruleus (37).

A 'J' shaped relationship has been reported between the volume of alcohol consumed and risk of dementia, where moderate drinkers are at lower dementia risk than abstainers and heavy drinkers (38). A systematic review and meta-analyses by Anstey et al. (39) found alcohol drinkers in late life have reduced risk of dementia. Follow-up ranged from 2 to 8 years. Meta-analyses were conducted on samples including 14,646 participants evaluated for Alzheimer dementia, 10,225 participants evaluated for vascular dementia and 11,875 followed for any type of dementia. Light to moderate drinkers were at lower risk for Alzheimer's dementia, Vascular dementia and any type of dementia compared to non-drinkers; Alzheimer's dementia (RR 0.72, 95% CI 0.61-0.86), Vascular dementia (RR 0.75, 95% CI 0.57-0.98), and any type of dementia (RR 0.74, 95% CI 0.61-0.91).

In summary, there is a strong body of evidence supporting the notion that lifestyle factors in adulthood are associated with neurodegeneration. The evidence encompasses a variety of study designs and covers a range of lifestyle factors including smoking, physical activity and alcohol. Further

exploration of the association between lifestyle and a specific biomarker of neurodegeneration - MRI, is therefore the focus of this thesis.

1.2 The importance of midlife risk factor modification to treat neurodegeneration

Pharmacological developments

The failure to develop new drug treatments to treat late life neurodegeneration, namely dementia, emphasises the importance of focusing on risk factor modification (40). There are four drugs approved for the treatment of Alzheimer's dementia. These drugs have clinical effects as demonstrated on the Alzheimer's Disease Assessment Scale – cognitive subscale (ADAS-cog) (41), however they do not modify the course of the disease. These four treatments are three cholinesterase inhibitors - donepezil, rivastigmine, galantamine and an N-methyl-D-aspartate (NMDA) receptor antagonist - memantine. Donepezil was approved by the Food and Drug Administration (FDA) in 1996, rivastigmine in 1998, galantamine in 2001, and memantine in 2003. There have been no new drugs approved for Alzheimer's dementia since 2003 (42). The drugs in development for Alzheimer's dementia according to the most advanced phase of study and main therapeutic properties are shown in Figure 2. This shows that 113 drugs were being investigated for Alzheimer's dementia in 2018 (40). Despite ongoing investigations, there has not been a significant advance in the development of drug treatments for dementia in 15 years.

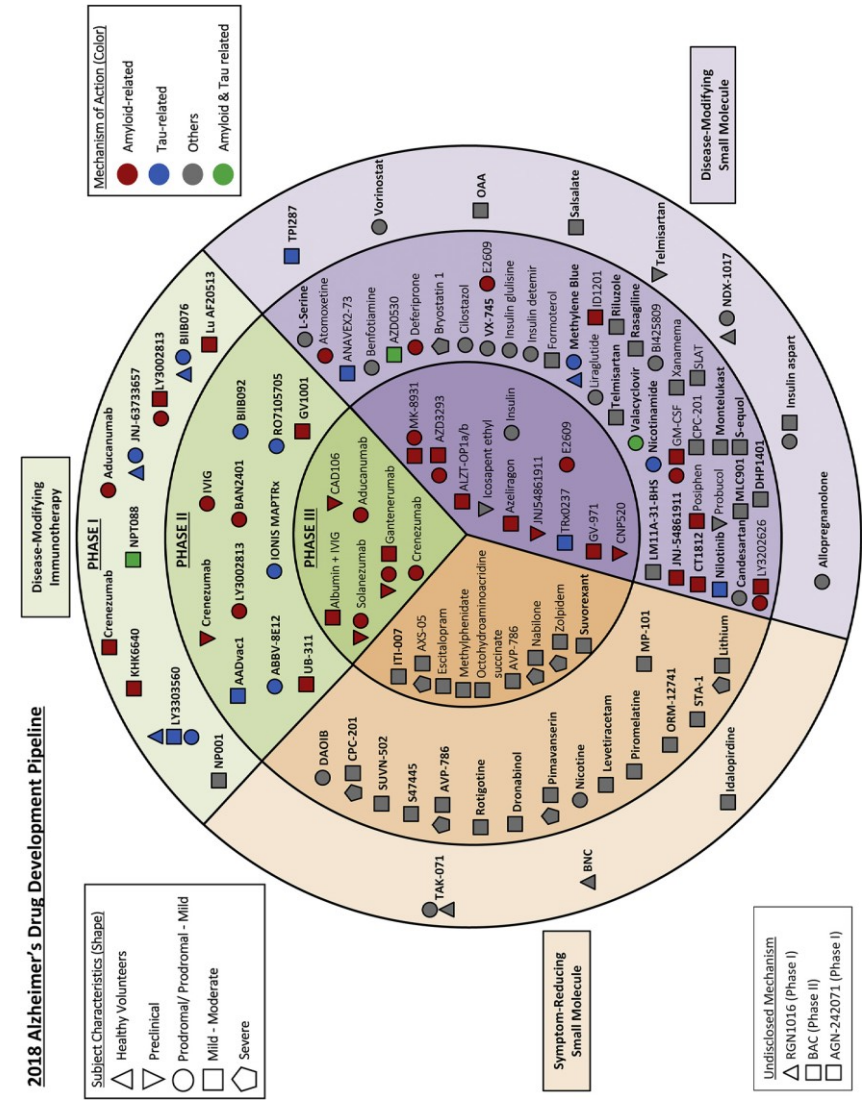

Figure 2: Drugs being investigated for Alzheimer's dementia therapy in 2018, reported according to the most advanced phase of study and main therapeutic properties (40)

Given the failure to develop pharmacological interventions, in addition to the strong body of evidence that demonstrates a relationship between lifestyle factors and neurodegeneration, three key recent studies, the preDIVA study (43), the Multidomain Alzheimer Preventive Trial (MAPT) (44) and the Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER) (45) have understandably sought to reduce dementia incidence through late life risk factor modification.

The preDIVA study

The preDIVA study aimed to reduce vascular risk factors to prevent dementia in a 6-year open-label, cluster-randomised control trial. Individuals aged 70–78 years were recruited through participating general practices in the Netherlands. The primary outcomes were cumulative incidence of dementia and disability score (Academic Medical Center Linear Disability Score (ALDS)) at 6 years of follow-up. Smoking habits, diet, physical activity, weight, and blood pressure were monitored and individually tailored lifestyle advice was given (43). Blood glucose and lipid concentrations were assessed every 2 years and if indicated, medication was given for hypertension, diabetes or dyslipidaemia. There were 3526 participants recruited and randomly assigned; 1890 participants to the intervention group and 1636 participants to the control group. The primary outcome data were obtained for 3454 (98%) participants and median follow-up was 6.7 years. Dementia incidence did not differ significantly between the intervention and usual care group. Dementia developed in 121 (7%) of 1853 participants in the intervention group and in 112 (7%) of 1601 participants in the control group

(hazard ratio (HR) 0·92, 95% CI 0·71–1·19). Mean ALDS scores measured during follow-up did not differ between groups (85·7 [SD 6·8] intervention vs 85·7 [SD 7·1] control). During the study, 309 (16%) of 1885 participants died in the intervention group, compared with 269 (16%) of 1634 participants in the control group (HR 0·98, 95% CI 0·80–1·18). Incident cardiovascular disease did not differ between groups, 273 (19%) of 1469 participants in the intervention group and 228 (17%) of 1307 participants in the control group (HR 1·06, 95% CI 0·86–1·31) (43). However, the trial did not specifically select a population with increased cardiovascular risk, potentially limiting the overall effect of the interventions. Furthermore the study population was aged 70–78 years, whereas the majority of evidence shows an association between midlife (40–60 years old) hypertension and dementia (46).

The Multidomain Alzheimer Preventive Trial

MAPT assessed the efficacy of supplementation with omega-3 fatty acid, a multidomain intervention consisting of nutritional counselling, physical exercise, cognitive stimulation or a combination of the two interventions on the change in cognitive function of participants aged 70 years and older for a period of 3 years (44). There were 1680 subjects, mean age 75.3 years and 64.8 % female enrolled by 13 memory clinics. Subjects were randomized into one of the following four groups: omega-3 supplementation alone, multidomain intervention alone, omega-3 plus multidomain intervention, or placebo. Participants underwent cognitive, functional and biological assessments at month 6, 12, 24 and 36. The primary endpoint was a change of memory function at three years, as assessed by the Free and Cued

Selective Reminding test (44). The results of MAPT showed no significant effects with any of the three treatment interventions compared with placebo on the primary outcome (47). In the context of increasing evidence that lifestyle and cardiovascular risk factors for neurodegeneration are prominent in midlife, it is noteworthy that the focus of this study was on individuals in late life (mean age 75 years old).

The Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability

The FINGER study (45), is a multicentre, multi-domain intervention double blind randomised control trial that is currently ongoing. To date, the trial has demonstrated that lifestyle intervention could maintain or even improve cognitive functioning in an elderly population. Between 2009 and 2011, 2654 individuals aged 60 -77 years old, were screened and 1260 individuals were entered into the study. Both the intervention group (n= 631) and the control group (n=629) received oral and written information and advice on healthy diet and level of physical, cognitive and social activities beneficial for vascular risk factors management and disability prevention from the study nurse. Additionally, the intervention group received nutritional guidance, physical training, cognitive training and social activity intervention in addition to intensive monitoring and management of metabolic and vascular risk factors. The nutritional intervention included individual sessions with a nutritionist. The physical training programme comprised of individually tailored, progressive muscle strength training and aerobic exercise program. The cognitive training targeted cognitive domains most sensitive to ageing

and with a central role in everyday situations (episodic memory, executive function, mental speed, and working memory). The training was competed in group sessions and individually using a computer-based program that was specially adapted for the FINGER from protocols previously shown to be effective in shorter term randomised controlled trials (48). The social activities were stimulated through the numerous group meetings of all intervention components.

The primary outcome was change in cognition as measured by the comprehensive neuropsychological test battery (NTB). Estimated mean change in NTB total Z score at 2 years was 0·20 (SD 0·51) in the intervention group and 0·16 (SD 0·51) in the control group. The between group difference in the NTB total score per year was 0·022 (95% CI 0·002-0·042, p=0·030). In the study, 153 (12%) individuals dropped out overall. Adverse events occurred in 46 (7%) participants in the intervention group compared with six (1%) participants in the control group; the most common adverse event was musculoskeletal pain. Findings from this large, long-term, randomised controlled trial suggest that a multidomain intervention could improve or maintain cognitive functioning in individuals in late life. However, it is important to bear in mind that, no evidence-based model exists to link marginal changes in cognitive functioning to modification of dementia incidence.

In summary, lifestyle modification may reduce an individual's risk of developing dementia; however, it is important to recognise that the effectiveness of a lifestyle modification could be dependent on the stage of

an individual's life at which it is made. The preDIVA study (43), MAPT (44) and FINGER (45) studies all focus on interventions that might have a limited impact in late life. Further research is needed to assess if the critical period for intervention is midlife.

Midlife lifestyle modification

There are a number of trials worldwide, collecting biomarker data on asymptomatic individuals at risk of late life dementia. This includes the ALFA project (49), the Adult Children Study (50), Wisconsin Registry for Alzheimer's Prevention (WRAP) (51), the European Prevention of Alzheimer's Dementia (EPAD) Longitudinal Cohort Study (LCS) (52) and the PREVENT Dementia cohort (53). Both the ALFA project (49) and the Adult Children Study (50) assess cognitively normal individuals aged between 45 and 74 years for biomarkers of Alzheimer's dementia. WRAP is a longitudinal observational cohort study of people age 40 – 65 years old. It is enriched with people with a parental history of probable Alzheimer's dementia (51). EPAD is a longitudinal cohort study of asymptomatic individuals aged 50 years old and over with no diagnosis of dementia. From the longitudinal cohort study, subjects will be selected for proof-of-concept trials for secondary prevention of Alzheimer's dementia (52). The PREVENT Dementia cohort is a prospective cohort study to identify midlife biomarkers of late life dementia (53). In the PREVENT Dementia cohort, midlife is defined as 40 -59 years old. In accordance with the PREVENT Dementia cohort, the same definition of midlife is used in my thesis. The use of this definition throughout my thesis gives the opportunity to directly use the systematic review findings to guide

the analysis of the PREVENT Dementia Cohort data. A comprehensive description of the PREVENT Dementia Cohort is given in Chapter 3.

This thesis will look at the association between lifestyle and neurodegeneration in midlife. Neuroimaging will be used as a biomarker for neurodegeneration as a clinical trial would not be feasible for the purposes of this thesis.

1.3 Functional Magnetic Resonance Imaging as a biomarker of neurodegeneration

As part of this thesis, a systematic review was undertaken to explore the relationship between lifestyle and neurodegeneration as expressed on fMRI in midlife. FMRI encompasses a family of non-invasive imaging techniques that provide information on multiple aspects of brain function and physiology. FMRI does not directly image neuronal activity; instead, it targets the detection of changes in its various physiological correlates such as Cerebral Blood Flow (CBF). FMRI is established as a marker of neurodegeneration and it has been used in the study of Cerebral Amyloid Angiopathy, Parkinson's disease, Amytropic Lateral Sclerosis and Dementia (54-57). The most studied neurodegenerative disease using fMRI is Alzheimer's disease (57). In the study of Alzheimer's disease, Asllani et al. (58) demonstrated that individuals with Alzheimer's disease showed a decrease in global grey matter CBF, compared to age matched cognitively normal adults with mean and standard deviation values of 36.1 ± 7.8 ml/100g per min for the Alzheimer's disease group and 62.4 ± 13 ml/100g per min for

the age matched cognitively normal adults group ($t = 6.05$, $P < 0.0001$). Additionally, alterations in resting CBF are associated with both first degree family history of Alzheimer disease and *APOE* ε4. First degree familial history of Alzheimer's disease can increase the risk of developing Alzheimer's disease by up to 10 fold and possessing the *APOE* ε4 allele has been shown to increase Alzheimer's disease risk by up to 8 fold (59, 60). Okonkwo et al. (61) demonstrated that cognitively intact individuals with a first degree family history of Alzheimer disease and *APOE* ε4 allele had reduced CBF in lateral frontal and superior parietal regions, hippocampus, precuneus and posterior cingulate. However, in a slightly younger group with first degree family history of Alzheimer disease and *APOE* ε4 allele, Fleisher et al. (62) showed increased hippocampal CBF compared to adults with no known risk factors for Alzheimer's disease. Chen et al. (57) proposed that in individuals at risk for Alzheimer's disease, elevated CBF represents a possible vascular regulatory mechanism to compensate for an increased need for glucose and oxygen in order to achieve a similar level of cognitive performance. Subsequently, lower CBF seen in older asymptomatic adults at higher risk, such as in those with a first degree family history of Alzheimer disease and *APOE* ε4 allele, suggests a breakdown in this early compensatory mechanism that may precipitate a decrease in neural activity and later degeneration and cognitive decline.

Hypothetical models of the development and progress of Alzheimer's disease suggest that fMRI is sensitive to the earliest neurodegenerative changes in the condition. In a hypothetical model of the dynamic biomarkers

of Alzheimer's disease, Jack et al. (63) proposed that biomarkers become abnormal in a temporally ordered manner. Initially cerebrospinal fluid (CSF) Amyloid-Beta and then amyloid PET followed by CSF tau and fluorodeoxyglucose PET abnormalities. In a later stage of Alzheimer's disease there are structural MRI (sMRI) abnormalities and then clinical symptoms become more evident. In an update to this model, Jack et al. (64) proposed that fMRI biomarkers may become abnormal before amyloid biomarkers become abnormal in Alzheimer's disease. An adapted version of the Jack et al. (63) model of the dynamic biomarkers of Alzheimer's disease, taking into account the potential early fMRI changes in Alzheimer's disease, is shown in Figure 3. The Jack et al. (63), model is complemented by the hypothetical model of the temporal ordering of physiological biomarkers of Alzheimer's disease by Wierenga et al (65), as shown in Figure 4. In this model, CBF changes that can be measured by fMRI occur early in the development of Alzheimer's disease. Changes in biomarkers measuring Amyloid-Beta, Tau mediated neuronal injury and dysfunction and Amyloid-Beta occur later in the development Alzheimer's disease. However, it is worth emphasizing that the models are based on assumptions rather than strong empirical data. Collection of empirical data is ongoing in studies such as the European Prevention of Alzheimer's Dementia (EPAD) Longitudinal Cohort Study (LCS) (52) and the PREVENT Dementia cohort (53).

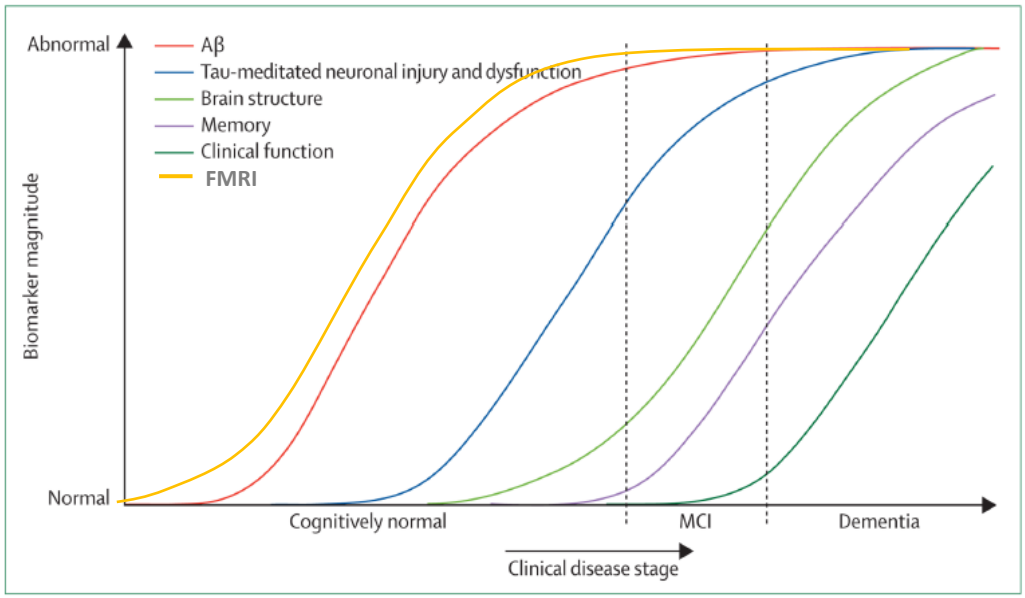

Figure 3: Adapted model of the dynamic biomarkers of Alzheimer's dementia.

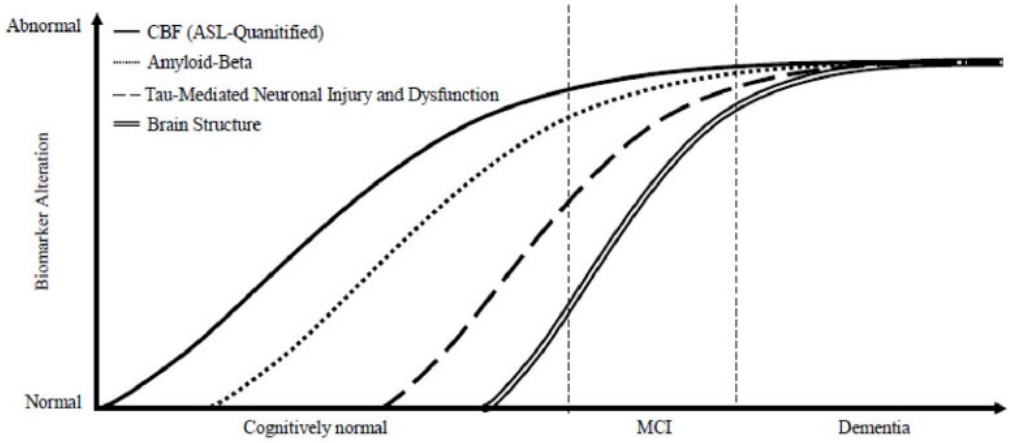

Figure 4: Hypothetical model of the temporal ordering of physiological biomarkers of Alzheimer's dementia (65).

CBF changes in Alzheimer's dementia are thought to initially result in hyperperfusion and later in hypoperfusion (65). The shift from hyperperfusion to hypoperfusion is consistent with the conclusions of a recent 8-year follow-up study of asymptomatic *APOE* ε4 carriers and controls who were 55 to 65 years of age at the time the study was initiated (66). At the initial examination, the *APOE* ε4 carriers showed significantly higher resting CBF values in a number of brain regions that are vulnerable to Alzheimer's dementia pathology. Eight years later the *APOE* ε4 carriers had significantly larger CBF reductions in the affected regions (66).

The capillary dysfunction hypothesis of Alzheimer's dementia developed by Ostergaard (67) offers a possible explanation for this biphasic response. It theorises that increases in the heterogeneity of capillary blood flow patterns occurs early in the preclinical stage of Alzheimer's dementia and requires increases in CBF to maintain adequate brain oxygenation. The mechanisms by which the initial hyperperfusion is then suppressed remains poorly understood. It is thought to involve tissue hypoxia that gradually develops when vasodilation can no longer replenish tissue oxygen during episodes of increased oxidative metabolism (67). However, this model is hypothetical and needs further testing.

1.4 What is lifestyle?

There is a lack of a clear definition of lifestyle in medical literature and more specifically in literature on neurodegeneration. In the Caerphilly Cohort Study, Elwood et al. (68) assessed the effect of lifestyle on chronic diseases

and dementia. In regard to lifestyle, Elwood et al. (68) noted the following, 'lifestyle and health-related behaviours are powerful determinants of morbidity and mortality worldwide, and unhealthy behaviours lie at the root of many chronic and disabling diseases. Major concerns focus on smoking, body mass, physical activity, diet and alcohol consumption, and the concept of a healthy lifestyle is usually defined with reference to combinations of these factors.' The ambiguity about what factors make up lifestyle, make it difficult to combine and summarise the findings from the current literature on lifestyle. In the Caerphilly Cohort Study (68), the factors which made up lifestyle were smoking status, Body mass index (BMI), portions of fruit and vegetables per day, regular exercise and alcohol intake. Contrastingly, in the World Alzheimer's Report on Dementia and Risk Reduction (35), the factors which made up lifestyle were smoking, physical activity, diet, alcohol and cognitive stimulation. In an interventional study to prevent dementia, in the FINGER study (45) a different interpretation of the key factors that make up lifestyle was used. In the FINGER study, four intensive lifestyle-based strategies (diet, exercise, cognitive training, and vascular disease management) were used to enhance cognition in an intervention group versus controls who received general health advice.

For my thesis, because of the lack of consensus in the published literature, I used the following definition of lifestyle from the World Health Organisation (WHO): 'Lifestyle is a way of living based on identifiable patterns of behaviour which are determined by the interplay between an individual's personal characteristics, social interactions, and socioeconomic

and environmental living conditions' (69). Based on this definition, amongst the six factors (smoking status, BMI, portions of fruit and vegetables per day, regular exercise and alcohol intake) which made up lifestyle in the Caerphilly Cohort Study (68), five would be in keeping with the WHO definition of lifestyle (smoking status, portions of fruit and vegetables per day, regular exercise and alcohol intake) and one factor, BMI, would not. BMI is not an identifiable pattern of behaviour, it is the consequence of an individual's lifestyle, in particular the frequency and intensity of physical activity and diet. All five lifestyle factors identified in the World Alzheimer's Report on Dementia and Risk Reduction (35) (smoking, physical activity, diet, alcohol and cognitive stimulation) would be in keeping with the WHO definition of lifestyle. Amongst the four interventions used in the FINGER study (45) (diet, exercise, cognitive training, and vascular disease management), three would be in accordance with the WHO definition of lifestyle (diet, exercise and cognitive training). Vascular disease management would not be a pattern of behaviour and it therefore conflicts with the WHO definition of lifestyle. My thesis will aim to explore all factors that are in accordance with the World Health Organisation (WHO) definition of lifestyle (69).

Statement of aim and objectives

Aim

To evaluate the relationship between lifestyle factors and neurodegeneration in midlife as expressed on arterial spin labelling and structural MRI.

Objectives

1. To undertake a systematic review of the relationship between lifestyle and neurodegeneration in midlife as expressed on functional MRI in the published literature.
2. Based on the findings of this systematic review, analytically assess the impact of lifestyle on cerebral blood flow in a midlife cohort.
3. Assess the impact of lifestyle on the structural MRI expression of neurodegeneration in a midlife cohort.

2 Systematic review of the relationship between lifestyle and neurodegeneration in midlife as expressed on functional magnetic resonance imaging

Chapter abstract

INTRODUCTION: Lifestyle factors may influence brain health in midlife. FMRI is a tool, widely used to investigate early changes in brain health, including neurodegeneration. In this systematic review, the relationship between lifestyle factors and neurodegeneration in midlife, as expressed using fMRI is evaluated.

METHODS: MEDLINE, EMBASE, and PsycINFO were searched by combining subject headings and free text terms adapted for each database. Articles were screened and their quality assessed independently by two reviewers before final inclusion in the review.

RESULTS: 4116 studies were screened and 29 were included in the review. Seven lifestyle factors were identified in this review; alcohol, cognitive training, excessive internet use, fasting, physical training, smoking and substance misuse.

DISCUSSION: Cognitive training and physical training appear to be associated with a neuroprotective effect, whereas alcohol misuse, smoking and substance misuse appear to be associated with neurodegeneration. Further research is required into the effects of excessive internet use and fasting.

2.1 Introduction

There is increasing recognition that neurodegenerative diseases, which manifest clinically as dementia in later life, have their origins in midlife, or even earlier (70). There is potentially a role for both pharmacological and non-pharmacological interventions such as lifestyle modification in the management of neurodegenerative diseases. In midlife, lifestyle modification may alter neurodegenerative disease progression and thereby reduce an individual's risk of dementia in later life (71). If this were the case, it is critical to identify which potentially modifiable lifestyle factors are associated with neurodegeneration in midlife. Recent research on cognitive, neuroimaging and biological markers suggest that changes in several parameters may well precede overt clinical symptoms by not just many years, but decades (53). FMRI is an established marker for neurodegenerative disease. It could also be of value in monitoring disease progression and response to interventions. Therefore, in the absence of any previous systematic reviews, I evaluated the relationship between lifestyle factors and neurodegeneration in midlife as expressed on fMRI in the published literature. A shortened version of this systematic review was published in 'Alzheimer's & Dementia: Translational Research & Clinical Interventions' on 3rd April 2018 (72). The published manuscript is available in Appendix A.

2.2 Methods

2.2.1 Identification of studies

MEDLINE, EMBASE, and PsycINFO were searched via the OVID platform on 5th December 2016 with no limits on search dates. Therefore, EMBASE was searched from 1980 to November 2016, MEDLINE was searched from 1946 to November 2016, PsycINFO from 1806 to November 2016. Additionally, there were no limits on language. A specific search strategy was constructed for each database using subject headings and free search terms. The search terms covered the areas of neuroimaging, lifestyle, and regional changes in cerebral metabolism or blood flow, blood volume, or oxygenation. Appendix B shows the search strategies for EMBASE, MEDLINE and PsycINFO.

2.2.2 Eligibility

The systematic review includes all published studies that assessed the relationship between lifestyle factors and neurodegeneration, neuroprotection or both as expressed on fMRI in midlife. A study was defined as having assessed individuals in midlife if it included individuals aged 40-59 years old or if two standard deviations around the mean reported age of participants fell within the 40-59 years age range. Neurodegeneration was defined as any pathological condition primarily affecting neurons (73). Neuroprotection was considered to be an effect that may result in salvage, recovery or regeneration of the nervous system, its cells, structure and function (74). The exposure in the review was lifestyle factors, as defined by the World Health Organisation: ‘Lifestyle is a way of living based on identifiable patterns of

behaviour which are determined by the interplay between an individual's personal characteristics, social interactions, and socioeconomic and environmental living conditions' (69). The outcomes in the systematic review are the numerical outcome measures derived from the fMRI scan. FMRI is a brain imaging technique that captures regional changes in cerebral metabolism or in blood flow, volume or oxygenation in response to task activation or during rest (75). The systematic review aimed to include studies using both resting-state and task-based fMRI experimental protocols and studies assessing the general population as well as those conducted in a general medical setting.

The inclusion criteria for the systematic review were:

i. Original human research study.
ii. Population includes a lifestyle factor in midlife.
iii. Study includes an fMRI outcome in midlife.

The exclusion criteria hierarchy for the systematic review were:

i. Insufficient information.
ii. Conference abstract.
iii. Not an original human research study.
iv. Study population has a diagnosis of dementia either in general or based on specific subtypes classified using standard diagnostic criteria, e.g. the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ARDRA) (76).
v. Study population does not include a lifestyle factor in midlife.

vi. Study population does not include individuals in midlife.

vii. The aim of the study is not to look at the effect of a lifestyle factor on fMRI outcome.

viii. FMRI outcome is not a proxy for neurodegeneration or neuroprotection.

2.2.3 Study selection and data collection

The titles and abstracts of all articles identified by the search were screened independently by two reviewers (Dr Hinesh Topiwala and Dr Lucy Stirland) against the inclusion and exclusion criteria with any disagreements in the final lists of included studies resolved by third party arbitration (Professor C. W. Ritchie). Potentially relevant articles were then retrieved and examined against the inclusion and exclusion criteria. Differences between reviewers' selections were again resolved by discussion. Data were extracted from included articles by one reviewer (Dr Hinesh Topiwala) on the number of study participants, mean age, standard deviation and age range of participants, study methodology and design and the key findings from the study related to this systematic review.

2.2.4 Quality of evidence

The quality of studies was assessed independently by two reviewers (Dr Hinesh Topiwala and Dr Lucy Stirland) using a modified Effective Public Health Practice Project Quality Assessment Tool (EPHPP) (77) tailored to the literature being assessed in this review. The EPHPP was modified by adding 'cross-sectional study' to the list of recognised study designs in section B. The EPHPP is available at: http://www.ephpp.ca/. The EPHPP tool has been

judged suitable for use in a systematic review and it is based on six component ratings: selection bias, study design, confounders, blinding, data collection method, withdrawals and dropouts (78). For each of these six components, a study is given a rating of strong, moderate or weak. The questions and key scoring guidelines for the modified EPHPP are shown in Table 1. On the scoring *pro forma*, a score is selected for each of the six component ratings and based on this, each individual study is given a global rating. When a study had no weak ratings, it was given a global quality rating of strong. When a study had one weak rating it was given a global quality rating of moderate. When a study had two or more weak ratings it was given a global quality rating of weak.

Table 1: Modified Effective Public Health Practice Project Quality Assessment Tool questionnaire and scoring system.

Component Rating	Question	Key scoring guidelines
Section A. Selection bias	Question 1. Are the individuals selected to participate in the study likely to be representative of the target population? Answer: 1. Very likely | 2. Somewhat likely | 3. Not likely | 4. Can't tell.	Participants are more likely to be representative of the target population if they are randomly selected from a comprehensive list of individuals in the target population (score very likely).
Section A. Selection bias	Question 2. What percentage of selected individuals agreed to participate? Answer: 1. 80 - 100% agreement | 2. 60 – 79% agreement | 3. Less than 60% agreement | 4. Not applicable | 5. Can't tell.	Refers to the percentage of subjects in the control and intervention groups that agreed to participate in the study before they were assigned to intervention or control groups.
Section A. Selection bias	Section rating: 1. Strong | 2. Moderate | 3. Weak.	Rating is strong when the selected individuals are very likely to be representative of the target population (Question 1 is 1) and there is greater than 80% participation (Question 2 is 1).
Section B. Study design	Question 3: Indicate the study design: Answer: 1. Randomised controlled trial | 2. Controlled clinical trial | 3. Cohort analytic (two group pre + post) 4 Case-control | 5. Cohort (one group pre + post) | 6. Interrupted time series | 7. Cross-sectional | 8. Other | 9. Can't tell.	The type of design is a good indicator of the extent of bias - in stronger designs, an equivalent control group is present and the allocation process is such that the investigators are unable to predict the sequence.
Section B. Study design	Section rating: 1. Strong | 2. Moderate | 3. Weak.	Rating is strong when the study describes a randomised controlled trial or case controlled trial.
Section C. Confounders	Question 4: Were there confounders between groups prior to the intervention? Answer: 1. Yes | 2. No | 3. Can't tell.	A confounder is a variable that is associated with the intervention or exposure and causally related to the outcome of interest.
Section C. Confounders	Question 5: If yes, indicate the percentage of relevant confounders that were controlled for? Answer: 1. 80 – 100% | 2. 60 – 79% | 3. Less than 60% | 4. Can't Tell.	Even in a robust study design, groups may not be balanced with respect to important variables prior to the intervention. The authors should indicate if confounders were controlled for.

Component Rating	Question	Key scoring guidelines
Section C. Confounders	Section rating: 1. Strong | 2. Moderate | 3. Weak.	Rating is strong when the study controlled for at least 80% of relevant confounders (question 1 is 2); or (question 2 is 1).
Section D. Blinding	Question 6: Were the outcome assessors aware of the intervention or exposure status of participants? Answer: 1. Yes | 2. No | 3. Can't tell.	Assessors should be described as blinded to which participants were in the control and intervention groups. The purpose of blinding the outcome assessors is to protect against detection bias.
Section D. Blinding	Question 7: Were the study participants aware of the research question? Answer: 1. Yes | 2. No | 3. Can't tell.	Study participants should be blinded to the research question. The purpose of blinding the participants is to protect against reporting bias.
Section D. Blinding	Section rating: 1. Strong | 2. Moderate | 3. Weak.	Rating is strong when the outcome assessor is not aware of the intervention status of participants (question 1 is 2); and the study participants are not aware of the research question (question 2 is 2).
Section E. Data collection methods	Question 8: Were data collection tools shown to be valid? Answer: 1. Yes | 2. No | 3. Can't tell.	Tools for primary outcome measures must be described as valid.
Section E. Data collection methods	Question 9: Were data collection tools shown to be reliable? Answer: 1. Yes | 2. No | 3. Can't tell.	Tools for primary outcome measures must be described as reliable.
Section E. Data collection methods	Section rating: 1. Strong | 2. Moderate | 3. Weak.	Rating is strong when the data collection tools have been shown to be valid (question 1 is 1) and the data collection tools have been shown to be reliable (question 2 is 1).
Section F. Withdrawals and dropouts	Question 10: Were withdrawals and drop-outs reported in terms of numbers and/or reasons per group? Answer: 1. Yes | 2. No | 3. Can't tell | 4. Not Applicable.	Score yes if the authors describe both the numbers and reasons for withdrawals and drop-outs.
Section F. Withdrawals and dropouts	Question 11: Indicate the percentage of participants completing the study. Answer: 1. 80 -100% | 2. 60 - 79% | 3. Less than 60% | 4. Can't tell | 5. Not Applicable.	The percentage of participants completing the study refers to the percentage of subjects remaining in the study at the final data collection period in all groups.
Section F. Withdrawals and dropouts	Section rating: 1. Strong | 2. Moderate | 3. Weak.	Rating is strong when withdrawals and drop-outs are reported (question 10 is 1) and the follow-up rate is 80% or greater (question 11 is 1).

Protocol and registration

The systematic review protocol was registered on the PROSPERO International Prospective Register of Systematic Reviews, registration number CRD42016045237, (https://www.crd.york.ac.uk/PROSPERO/) on 5th August 2016. Please see Appendix C for the systematic review protocol.

2.3 Results

2.3.1 PRISMA flow diagram

The PRISMA diagram (Figure 5) for the screening and selection of studies, shows 4116 records were identified through database searches. Following deduplication and title and abstract screening, 255 full-text articles were assessed for eligibility. After excluding 226 articles, a total of 29 articles were included in the systematic review.

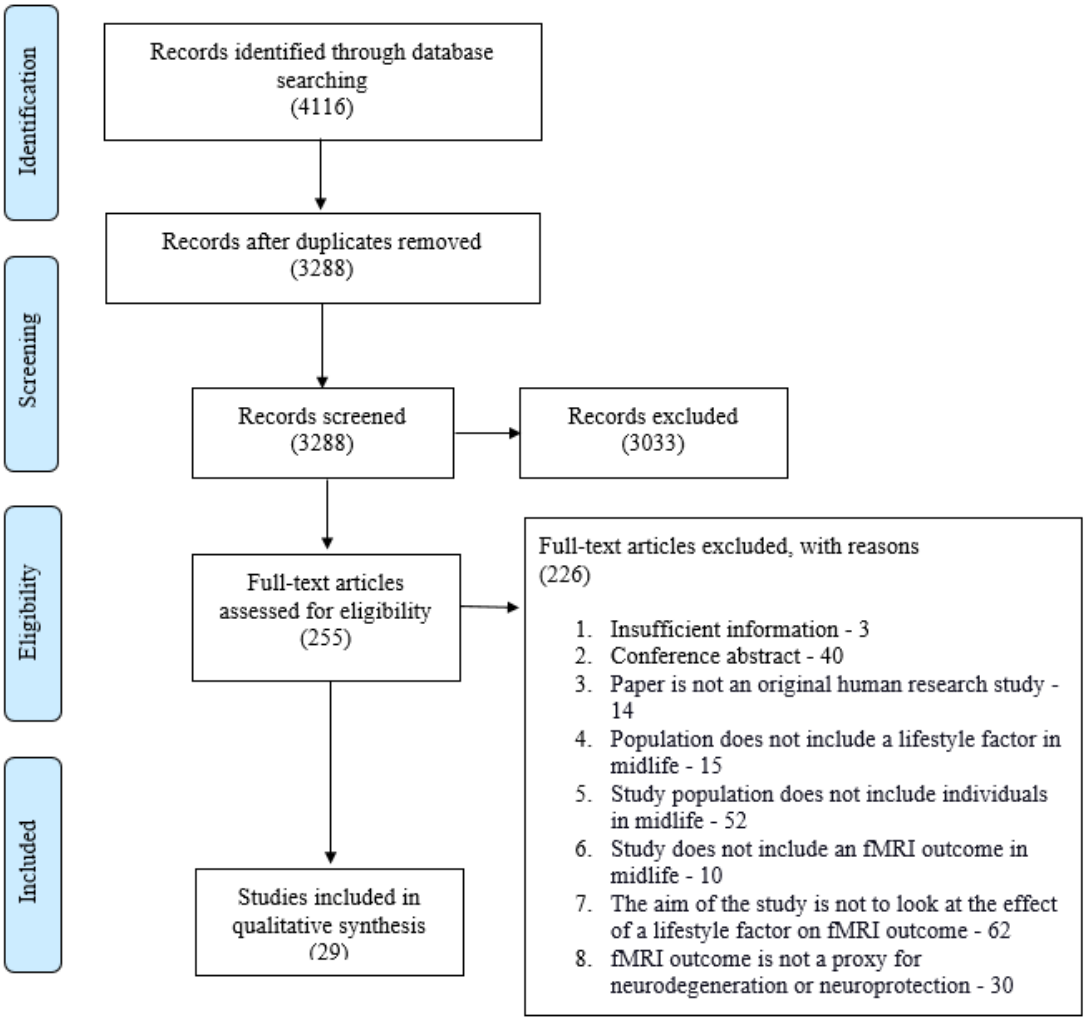

Figure 5: PRISMA diagram showing the selection of studies from search to inclusion.

2.3.2 Articles excluded at full-text screening

Of the 226 articles excluded during full text screening, three articles were excluded for having insufficient information. For these articles, the authors were contacted with a request for further information. The authors did not provide sufficient information to determine if the articles were eligible for inclusion in this systematic review. There were 40 conference abstracts identified by the systematic review search strategy. The conference abstracts had minimal information on methods and results; they were therefore excluded. The search strategy also identified letters, reports and animal studies, which led to 14 articles being excluded for not being an original human research study. This systematic review focuses on midlife dementia prevention, therefore 15 articles were excluded as the study population did not include a lifestyle factor in midlife, 52 were excluded as the study population did not include individuals in midlife and 10 were excluded as the study population did not include an fMRI outcome in midlife. There were 62 articles that were excluded as the aim of the study was not to look at the effect of a lifestyle factor on fMRI outcome. For example, Blum et al. (79) assessed the effect of an anti-craving treatment, KB220ZTM, in individuals with a heroin addiction. There were 30 studies that were excluded as the fMRI outcome in the study was not a proxy for neurodegeneration or neuroprotection. For example, Asensio et al. (80) evaluated relapse behaviour in substance misusers, by measuring acute changes in neural responsivity as expressed on fMRI, in response to erotic, unpleasant and

neutral images. The reasons that individual studies were excluded at full text screening are shown in Appendix D.

2.3.3 Studies included in qualitative synthesis

Of the 29 articles included at full text screening, six studies reported on the relationship between alcohol and neurodegeneration in midlife as expressed on fMRI, three studies reported on cognitive training, one study reported on excessive internet use, one study on fasting, seven studies on physical training, three studies on smoking and 12 studies reported on substance misuse. The lifestyle factors assessed by the studies included in the qualitative synthesis is shown on the Venn diagram in Figure 6. This highlights that some of the studies looked at more than one lifestyle factor. Of the 29 articles included in the systematic review, two studies looked at cognitive training and physical training, one study looked at alcohol and excessive internet use and one study looked at alcohol and smoking.

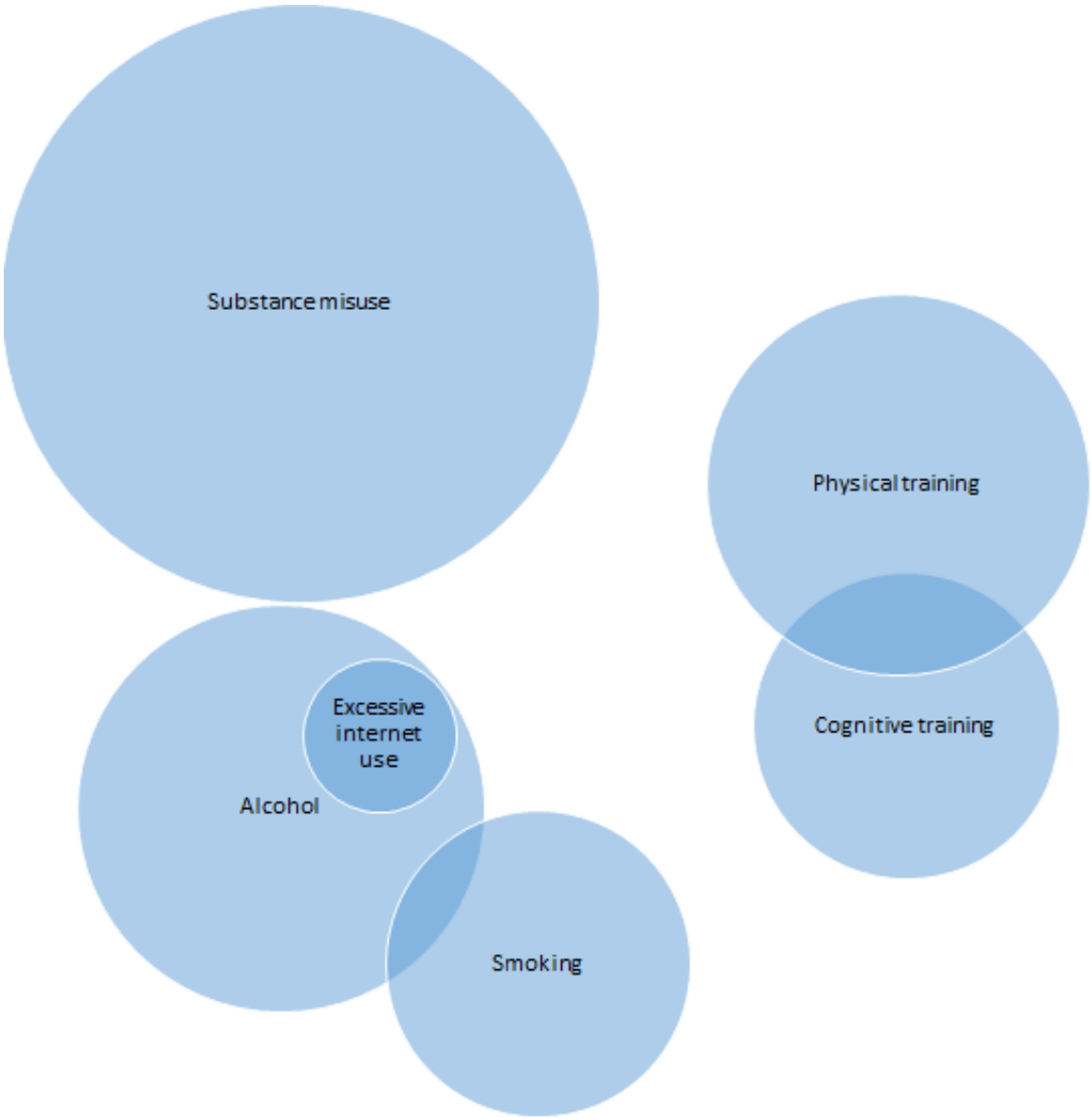

Figure 6: Venn diagram of the lifestyle factors assessed by the studies included in the qualitative synthesis. The size of the circles represents the number of studies identified by the systematic review. The overlap between the circles represents studies that assessed more than one lifestyle factor.

The EPHPP global quality assessment rating of the studies was weak for 9 studies, moderate for 9 studies and strong for 11 studies. Table 2 shows the EPHPP component ratings and global quality assessment ratings for studies included in the systematic review. It is noteworthy, that none of the studies were rated as strong for the selection bias component rating or blinding component rating. The selection bias component rating is strong if the participants in the study are very likely to be representative of the target population. For this to be the case, participants would need to be randomly selected from a comprehensive list of individuals in the target population and the percentage of individuals that agreed to participant in the study needs to be greater than 80%. This information was not stated for studies identified by this systematic review. The blinding component rating is strong if the outcome assessor is not aware of the intervention status of participants and the study participants are not aware of the research question. This information was not stated, for any of the studies identified in the systematic review.

Table 2: Modified Effective Public Health Practice Project Quality Assessment Tool global rating for individual studies reporting on the relationship between lifestyle factors and neurodegeneration in midlife as expressed on fMRI.

Study name	Selection bias component rating	Study design component rating	Confounders component rating	Blinding component rating	Data collection method component rating	Withdrawals and dropouts component rating	Global rating
Bednarski et al. (81)	3	2	1	2	1	2	2
Belaich et al. (82)	3	2	NA	2	1	3	3
Castelluccio et al. (83)	3	2	3	2	1	2	3
Chapman et al. (84)	2	1	1	2	1	1	1
Chapman et al. (85)	2	1	1	2	1	1	1
Chapman et al. (86)	2	1	1	2	1	2	1
Durazzo et al. (87)	3	2	1	2	3	2	3
Gazdzinski et al. (88)	2	2	3	2	1	2	2
Hart et al. (89)	2	2	1	2	1	2	1
Hermann et al. (90)	3	2	3	2	1	3	3
Hotting et al. (91)	2	1	1	2	1	2	1
Hu et al. (92)	3	2	1	2	1	2	2
Ide et al. (93)	3	2	1	2	1	2	2
Jiang et al. (94)	2	2	1	2	1	2	1
Kelly et al. (95)	2	2	1	2	1	2	1
Kim et al. (96)	2	2	3	2	1	2	2
Lee et al. (97)	3	2	3	2	3	2	3
Liu et al. (98)	3	2	3	2	3	2	3
MacIntosh et al. (99)	2	2	NA	2	3	1	2
May et al. (100)	3	2	3	2	1	2	3
McFadden et al. (101)	3	2	NA	2	1	1	2
Mitchell et al. (102)	2	2	2	2	1	1	1
Mon et al. (103)	2	2	1	2	1	2	1
Murray et al. (104)	2	2	2	2	1	2	1
Rogers et al. (105)	2	2	3	2	3	2	3
Sullivan et al. (106)	2	2	3	2	1	2	2
Wang et al. (107)	2	2	3	2	1	2	2
Weiland et al. (108)	3	2	3	2	1	2	3
Xu et al. (109)	2	2	NA	2	1	2	1

1 = Strong, 2 = Moderate, 3 = Weak, NA = Not applicable.

Table 3 summarises the lifestyle factors and global quality assessment ratings of the 29 articles included in the systematic review. The table is arranged alphabetically by lifestyle factor. Of the six studies that assessed alcohol, three studies had a rating of weak and three studies had a rating of moderate. The three studies that assessed cognitive training all had a rating of strong, the one study that assessed excessive internet use had a quality rating of moderate and the one study that assessed fasting had a quality rating of weak. Of the seven studies that assessed physical training, two studies had a rating of moderate and five studies had a rating of strong. Of the three studies that assessed smoking, one study had a rating of weak, one study had a rating of moderate and one study had a rating of strong. Of the twelve studies that assessed substance misuse, four studies had a rating of weak, four studies had a rating of moderate and four studies had a rating of strong.

Table 3: Summary of identified lifestyle factors and modified Effective Public Health Practice Project Quality Assessment Tool global ratings for studies reporting on the relationship between lifestyle factors and neurodegeneration in midlife as expressed on fMRI.

Lifestyle Factor	Number of studies	Global rating
Alcohol	6	Weak: 3 studies Moderate: 3 studies
Cognitive training	3	Strong: 3 studies
Excessive internet use	1	Moderate: 1 study
Fasting	1	Weak: 1 study
Physical training	7	Moderate: 2 studies Strong: 5 studies
Smoking	3	Weak: 1 study Moderate: 1 study Strong: 1 study
Substance misuse	12	Weak: 4 studies Moderate: 4 studies Strong: 4 studies

Table 4 shows the demographic information for the individual studies reporting on the relationship between lifestyle factors and neurodegeneration in midlife as expressed on fMRI. From the table it is notable that other than the study by Weiland et al. (108) which had 470 participants, all the studies had a relatively small number of participants, ranging from 12 to 163. For 16 or the 29 studies identified by this review, the mean age of individuals was outwith the 40-59 years old category, however two standard deviations around the mean reported age of participants fell within the 40-59 years old category.

Table 4: **Demographic information for the individual studies reporting on the relationship between lifestyle factors and neurodegeneration in midlife as expressed on fMRI.**

Study	Lifestyle Factor	N	Mean Age (SD) / Range
Bednarski et al. (81)	Substance Misuse	50 (cocaine dependence 23/ healthy controls 27).	Cocaine dependence: 36.2 (6.3) / NA. Healthy controls: 34.9 (6.5) / NA.
Belaich et al. (82)	Fasting	6 males.	41 (NA) / 34 – 48.
Castelluccio et al. (83)	Substance Misuse	94 (cocaine users 30/ former users 29/ healthy controls 35).	Cocaine users: 37.6 (7.3) / 21-45. Former users: 40.3 (6.4) / 22-50. Healthy controls: 35.8 (10) / 21-58.
Chapman et al. (84)	Physical Training	37 (physical training 18/ control 19).	64 (3.9) / 57-75.
Chapman et al. (85)	Cognitive Training	37 (cognitive training 18/ control 19).	62.9 (3.6) / 56-71.
Chapman et al. (86)	Physical and Cognitive Training	36 (physical training 18/ cognitive training 18).	Physical training: 64 (4.3) / 56-75. Cognitive training: 61.8 (3.3) / 56-75.
Durazzo et al. (87)	Smoking	61 (smokers 34/ non-smokers 27).	Smokers: 47.3 (10.5) / NA. Non-smokers: 47.3 (11.9) / NA.
Gazdzinski et al. (88)	Alcohol and Smoking	48 (non-smoking light drinkers 19/ non-smoking alcoholics 10/ smoking alcoholics 19).	Non-smoking light drinkers: 47 (7.9) / 26-66. Non-smoking alcoholics: 50.9 (10) / 26-66. Smoking alcoholics: 48 (9.9) / 26-66.

Study	Lifestyle Factor	N	Mean Age (SD) / Range
Hart et al. (89)	Physical Training	52 (ex-NFL players 26/ healthy controls 26).	Ex-NFL players: 61.8 (NA) / 41-79. Healthy controls: 60.1 (NA) / 41-79.
Hermann et al. (90)	Alcohol	18 (alcohol dependence 9/ healthy volunteers 9).	Alcohol dependence: 40.2 (5.6) / NA. Healthy control: 41.8 (13.2) / NA.
Hotting et al. (91)	Physical and Cognitive Training	33 (cycling and spatial 8, cycling and perceptual 8, stretching and spatial 9, stretching and perceptual 8).	Cycling and spatial: 50.25 (4.2) / 40-55. Cycling and perceptual: 49 (4.28) / 40-55. Stretching and spatial: 50.22 (2.91) / 40-55. Stretching and perceptual: 46 (3.89) / 40-55.
Hu et al. (92)	Substance Misuse	112 (cocaine users 56/ healthy controls 56).	Cocaine users: 39.86 (6.71) / NA. Healthy controls: 38.70 (10.9) / NA.
Ide et al. (93)	Substance Misuse	163 (cocaine dependent 75/ healthy controls 88).	Cocaine dependent: 39.9 (7.6) / NA. Healthy controls: 38.7 (10.9) / NA.
Jiang et al. (94)	Substance Misuse	48 (chronic heroin users 24 / normal controls 24).	Chronic heroin users: 35.67 (5.66) / NA. Normal controls: 35.38 (6.02) / NA.
Kelly et al. (95)	Substance Misuse	49 (cocaine dependent 25/ healthy comparisons 24).	Cocaine dependent adults: 35.0 (8.8) / NA. Healthy comparisons: 35.1 (7.5) / NA.
Kim et al. (96)	Excessive Internet and Alcohol Use	45 (internet gaming disorder 16 / alcohol use disorder 14 / healthy controls 15).	Internet gaming disorder: 21.63 (5.92) / NA. Alcohol use disorder: 28.64 (5.92) / NA. Healthy controls: 25.40 (5.92) / NA.
Lee et al. (97)	Substance Misuse	23 (chronic cocaine abusers 13/ healthy controls 10).	Chronic cocaine abusers: 38 (6) / 28-45. Healthy controls: 36 (6) / 27-44.
MacIntosh et al. (99)	Physical Training	30 men with coronary artery disease.	65 (7) / 55-80.
May et al. (100)	Substance Misuse	42 (recently abstinent methamphetamine dependent 25 /healthy controls 17).	Abstinent methamphetamine dependent individuals: 38.84 (9.16) / NA. Healthy controls: 38.77 (9.40) / NA.
McFadden et al. (101)	Physical Training	12 overweight/ obese adults.	38.2 (9.5) / NA.

Study	Lifestyle Factor	N	Mean Age (SD) / Range
Mitchell et al. (102)	Substance Misuse	32 (cocaine dependence 16/ healthy controls 16).	Cocaine dependence: 39 (10.4) / NA. Healthy controls: 40 (7.4) / NA.
Mon et al. (103)	Smoking	69 (non-smoking light drinker 28, non-smoking alcoholic 19, smoking alcoholic 22).	Non-smoking light drinker: 44 (8.2) / 28-68. Non-smoking alcoholic: 52.1 (9.4) / 28-68. Smoking alcoholic: 47.8 (9.2) / 28-68.
Murray et al. (104)	Substance Misuse	77 (alcohol dependent 26, polysubstance use 20, light or non-drinkers 31).	Alcohol dependent: 54 (10) / NA. Polysubstance use: 45 (9) / NA. Light or non-drinkers: 47 (11) / NA.
Rogers et al. (105)	Alcohol	20 (alcoholic 10/ healthy controls 10).	Alcoholic: 43 (12) / 18-70. Control: 40 (13) / 18-70.
Sullivan et al. (106)	Alcohol	24 (alcoholics 12/ control subjects 12).	Alcoholics: 45.7 (4.4) / 38-54. Control subjects: 46.3 (5.2) / 38-54.
Wang et al. (107)	Substance Misuse	39 (cocaine addict 20/ healthy controls 19).	Cocaine addict: 42.15 (4.3) / NA. Healthy controls: 39.9 (4.5) / NA.
Weiland et al. (108)	Alcohol	470 (problematic alcohol use 383/ controls 87).	Problematic alcohol use: 31.1 (9.3) / 21-56. Controls: 25.8 (8.3) / 21-53.3.
Xu et al. (109)	Physical Training	59 healthy adults.	66.68 (9.63) / NA.

NA = Not available, SD = Standard Deviation.

Table 5 outlines the key features of the methodology of the individual studies reporting on the relationship between lifestyle factors and neurodegeneration in midlife as expressed on fMRI. Of the 29 studies included in the systematic review, 23 used a cross-sectional in design, two were clinical controlled trials, two were cohort studies and one was a randomised control trial. Furthermore 18 of the 29 studies used resting state fMRI and 11 of the 29 studies used a task based fMRI for neuroimaging. A

wide variety of statistical methods were used to analyse the data generated by the studies included in the systematic review.

Table 5: Key features of the methodology of the individual studies reporting on the relationship between lifestyle factors and neurodegeneration in midlife as expressed on fMRI.

Study	Lifestyle Factor	Key features of methodology
Bednarski et al. (81)	Substance Misuse	Study type: Cross-sectional study. fMRI type: Task-based. Statistical method: One sample & two sample t-tests.
Belaich et al. (82)	Fasting	Study type: Cross-sectional study. fMRI type: Task-based. Statistical method: ANOVA test.
Castelluccio et al. (83)	Substance Misuse	Study type: Cross-sectional study. fMRI type: Task-based. Statistical method: Regression analysis.
Chapman et al. (84)	Physical Training	Study type: Controlled clinical trial. fMRI type: Resting-state. Statistical method: General statistical linear model.
Chapman et al. (85)	Cognitive Training	Study type: Controlled clinical trial. fMRI type: Resting-state. Statistical method: General statistical linear model.
Chapman et al. (86)	Physical or Cognitive Training	Study type: Randomised control trial. fMRI type: Resting-state. Statistical method: General statistical linear model.
Durazzo et al. (87)	Smoking	Study type: Cross-sectional study. fMRI type: Resting-state. Statistical method: Multivariate analysis of covariance (MANCOVA).
Gazdzinski et al. (88)	Alcohol and Smoking	Study type: Cross-sectional study. fMRI type: Resting-state. Statistical method: MANOVA; Wilks' λ.
Hart et al. (89)	Physical Training	Study type: Cross-sectional study. fMRI type: Resting-state. Statistical method: NA.
Hermann et al. (90)	Alcohol	Study type: Cross-sectional study. fMRI type: Task-based. Statistical method: General statistical linear model.
Hotting et al. (91)	Physical and Cognitive Training	Study type: Controlled clinical trial. fMRI type: Task-based. Statistical method: One and two sample t-tests, a flexible factorial model and full factorial model.
Hu et al. (92)	Substance Misuse	Study type: Cross-sectional study. fMRI type: Resting-state. Statistical method: ANOVA test.
Ide et al. (93)	Substance Misuse	Study type: Cross-sectional study. fMRI type: Task-based. Statistical method: Two sample t-tests and multiple regression.
Jiang et al. (94)	Substance Misuse	Study type: Cross-sectional study. fMRI type: Resting-state. Statistical method: Two sample t-tests and a Pearson correlation analysis.

Study	Lifestyle Factor	Key features of methodology
Kelly et al. (95)	Substance Misuse	Study type: Cross-sectional study. fMRI type: Resting-state. Statistical method: NA.
Kim et al. (96)	Excessive Internet & Alcohol Use	Study type: Cross-sectional study. fMRI type: Resting-state. Statistical method: two-sample t-tests.
Lee et al. (97)	Substance Misuse	Study type: Cross-sectional study. fMRI type: Task-based. Statistical method: ANOVA, Post hoc t-test, Pearson product moment correlations.
Liu et al. (98)	Substance Misuse	Study type: Cross-sectional study. fMRI type: Resting-state. Statistical method: Kolmogorov–Smirnov test, Mann–Whitney U test, linear regression.
MacIntosh et al. (99)	Physical Training	Study type: Cohort study. fMRI type: Resting-state. Statistical method: linear regression analysis.
May et al. (100)	Substance Misuse	Study type: Cross-sectional Study. fMRI type: Task-based. Statistical method: Linear mixed effects analysis.
McFadden et al. (101)	Physical Training	Study type: Cohort Study. fMRI type: Resting-state. Statistical method: t- test.
Mitchell et al. (102)	Substance Misuse	Study type: Cross-sectional study. fMRI type: Task-based. Statistical method: two-sample t-tests.
Mon et al. (103)	Smoking	Study type: Cross-sectional study. fMRI type: Resting-state. Statistical method: Generalized linear model.
Murray et al. (104)	Substance Misuse	Study type: Cross-sectional study. fMRI type: Resting-state. Statistical method: Analyses of covariance (ANCOVA).
Rogers et al. (105)	Alcohol	Study type: Cross-sectional study. fMRI type: Task-based. Statistical method: Two-sample t-test.
Sullivan et al. (106)	Alcohol	Study type: Cross-sectional study. fMRI type: Task-based. Statistical method: ANOVA.
Wang et al. (107)	Substance Misuse	Study type: Cross-sectional study. fMRI Type: Resting-state. Statistical method: Two sample t-test and simple regression.
Weiland et al. (108)	Alcohol	Study type: Cross-sectional Study. fMRI Type: Resting-state. Statistical method: Multivariate analysis of variance (MANCOVA)
Xu et al. (109)	Physical Training	Study type: Cross-sectional study. fMRI type: Resting-state. Statistical method: General linear model.

ANOVA = Analysis of the Variance, fMRI = functional Magnetic Resonance Imaging, MANOVA = Multivariate analyses of variance, NA = Not available.

Alcohol

Table 6 outlines the key findings of the individual studies reporting on the relationship between alcohol and neurodegeneration in midlife as expressed on fMRI. Of the 6 studies looking at the effect of alcohol as expressed on fMRI, 3 used a task-based fMRI protocol (90, 105, 106) and 3 studies used a resting-state protocol (88, 96, 108). Of the resting-state studies, Gazdzinski et al. (88) demonstrated evidence that concurrent alcohol dependence and chronic cigarette smoking is associated with regional grey matter hypoperfusion. Kim et al. (96) showed increased regional homogeneity (ReHo) in the posterior cingulate cortex (PCC) executive control, basal ganglia, and primary visual networks. Weiland et al. (108) found significantly lower network connectivity strength than controls in the left executive control, basal ganglia, and primary visual networks. For the task-based studies, Hermann et al. (110) described significantly lower blood oxygen dependent (BOLD) signal in an extended bilateral occipital area as compared with healthy controls during a visual and acoustic simulation task. Rogers et al. (105) described a pattern in recently abstinent alcoholic patients of specific deficits in functional connectivity and recruitment of additional brain regions for the performance of a simple finger-tapping task. Sullivan et al. (106) demonstrated that alcoholics have selective differences from control subjects in the cerebral blood flow (CBF) pattern in the anterior precuneus and CBF level in the insula, a hub of the salience network.

Table 6: Key findings of the individual studies reporting on the relationship between lifestyle factors and neurodegeneration in midlife as expressed on fMRI.

Study	Lifestyle Factor	Key findings
Gazdzinski et al. (88)	Alcohol and Smoking	Alcoholics, as a group, showed lower frontal grey matter perfusion and lower parietal grey matter perfusion than non-smoking light drinkers. In smoking alcoholics, a higher number of cigarettes smoked per day was associated with lower perfusion.
Hermann et al. (90)	Alcohol	Alcohol dependent patients showed a significantly lower BOLD signal in an extended bilateral occipital area as compared with healthy controls.
Kim et al. (96)	Excessive Internet & Alcohol Use	There were distinctive functional changes in the resting-state of patients with internet gaming disorder, in addition to common ReHo changes in the internet gaming disorder and alcohol use disorder group.
Rogers et al. (105)	Alcohol	Recently abstinent alcoholic patients showed deficits in functional connectivity and recruitment of additional brain regions for the performance of a simple finger-tapping task.
Sullivan et al. (106)	Alcohol	Alcoholics showed selective differences from control subjects in the CBF pattern in the anterior precuneus and CBF level in the insula, a hub of the salience network.
Weiland et al. (108)	Alcohol	Individuals with problematic alcohol use had significantly lower network connectivity strength than controls in the left executive control network, basal ganglia, and primary visual networks.

BOLD = Blood Oxygenation Level Dependent, fMRI = functional Magnetic Resonance Imaging, ReHo = regional homogeneity.

Cognitive training

Table 7 outlines the key findings of the individual studies reporting on the relationship between cognitive training and neurodegeneration in midlife as expressed on fMRI. There were 3 studies that looked at the effect of cognitive training as expressed on fMRI. Chapman et al. (85) examined changes in brain health, pre, mid, and post training in 37 adults who received 12 weeks' strategy based cognitive training versus a control group. They found increases in global and regional CBF, particularly in the default mode network and the central executive network. Additionally, they found greater connectivity in these same networks. In a follow-up, randomised control trial

comparing the effects of two training protocols, cognitive training and physical training, Chapman et al. (86) describe preliminary evidence that increased cognitive and physical activity improves brain health in distinct ways. Cognitive reasoning training enhanced frontal networks shown to be integral to top-down cognitive control and brain resilience. In a controlled clinical trial, Hotting et al. (91) compared effects of cognitive training (spatial vs. perceptual training) and physical training (endurance training vs. non-endurance training) on spatial learning in 33 adults. Spatial learning was assessed with a virtual maze task, and at the same time neural correlates were measured with fMRI. Only spatial training improved performance in the maze task. This improvement was accompanied by a decrease in frontal and temporal lobe activity.

Table 7: Key findings of the individual studies reporting on the relationship between lifestyle factors and neurodegeneration in midlife as expressed on fMRI.

Study	Lifestyle Factor	Key findings
Chapman et al. (85)	Cognitive Training	There was increased global and regional CBF particularly in the default mode network and the central executive network in the cognitive training group.
Chapman et al. (86)	Physical or Cognitive Training	There were multiple distinct changes on fMRI that suggest that aerobic exercise and cognitive reasoning training contribute differentially to brain health.
Hotting et al. (91)	Physical and Cognitive Training	Participants of the spatial training group showed lower activity than participants of the perceptual training group in a network of brain regions associated with spatial learning, including the hippocampus and parahippocampal gyrus.

CBF = Cerebral Blood Flow, fMRI = functional Magnetic Resonance Imaging.

Excessive internet use

Kim et al. (96) assessed the resting-state brain of individuals who were diagnosed with internet gaming disorder (IGD), as defined by DSM-V (111), who scored over 70 on Young's Internet Addiction Test (112) and who spent who spent more than four hours per day and 30 hours per week using the internet. These individuals were compared to patients with alcohol use disorder (AUD) and healthy controls (HC). Using ReHo measures, Kim et al. (96) found increased ReHo in the PCC of the IGD and AUD groups, and decreased ReHo in the right superior temporal gyrus of those with IGD, compared with the AUD and HC group. Scores on internet addiction severity were positively correlated with ReHo in the medial frontal cortex, precuneus/PCC, and left inferior temporal cortex among those with IGD.

Fasting

Belaich et al. (82) assessed the effect of daytime fasting on six healthy male volunteers during Ramadan. Two task-based BOLD-fMRI scan sessions were performed, the first scan between the fifth and tenth days preceding the start of fasting and the second scan between the 25th and 28th day of the fasting month. The study demonstrated a significant difference in the activated brain area between maximal BOLD-fMRI signal before and during fasting. Additionally, there was an increase in the volume of the activated brain area in all subjects during fasting. The authors suggested that the results of the study demonstrate an activation of adaptive mechanisms that may be beneficial for neuronal health and plasticity.

Physical training

Table 8 outlines the key findings of the individual studies reporting on the relationship between physical training and neurodegeneration in midlife as expressed on fMRI. There were five interventional and two observational studies that looked at the effect of physical training on brain health in midlife, as expressed on fMRI. Chapman et al. (113) assessed brain blood flow in 37 sedentary adults, in a clinical control trial of physical training versus a wait-list control group. Over 12 weeks, the physical training group received supervised aerobic exercise for three one-hour sessions per week. The study found higher resting CBF in the anterior cingulate region in the physical training group as compared to the control group from baseline to post-training. In a follow-up randomised control study, Chapman et al. (86) compared the effects of two training protocols, cognitive training and physical training on brain function. The study indicates that increased cognitive and physical activity improves brain health in distinct ways. Aerobic exercise improved CBF flow in hippocampi of those with memory gains.

MacIntosh et al. (99) assessed 30 men with Coronary Artery Disease, before and after a six-month cardiac rehabilitation programme consisting of aerobic and resistance training. CBF was associated with fitness level at baseline and greater fitness gains with exercise. McFadden et al. (101) assessed the effects of a six-month exercise intervention on intrinsic activity in the default mode network (DMN) in 12 overweight or obese individuals. The intervention was associated with a reduction in DMN activity in the

precuneus. This finding is thought to represent a normalisation of DMN function secondary to exercise.

In a controlled clinical trial, Hotting et al. (91) assessed the effects of cognitive training (spatial vs. perceptual training) and physical training (endurance training vs. non-endurance training) on spatial learning and associated brain activation in 33 adults. Spatial learning was assessed with a virtual maze task, and at the same time neural correlates were measured with fMRI. The two physical intervention groups did not improve performance in the maze task.

The findings of the two observational studies related to physical training focused on specific population groups. Hart el al. (89) assessed ageing former National Football League (NFL) players. They found that cognitive deficits and depression appear to be more common in ageing former NFL players than healthy controls. Additionally, altered CBF patterns are concordant with brain regions associated with abnormal findings of neuropsychological testing in ageing former NFL players. It is noteworthy, that of the 34 ex-NFL players included in the study, 32 reported a history of at least one concussion and 26 undertook neuroimaging; 8 were claustrophobic and did not undergo an MRI scan. In a cross sectional study of 59 adults recruited through a Rhode Island newspapers and an outpatient cardiology office, Xu et al. (109) found that women who engaged in strength training (weight lifting or calisthenics) at least once per week exhibited significantly greater cerebrovascular perfusion than women who did not.

Table 8: Key findings of the individual studies reporting on the relationship between lifestyle factors and neurodegeneration in midlife as expressed on fMRI.

Study	Lifestyle Factor	Key findings
Chapman et al. (84)	Physical Training	There is higher resting CBF in the anterior cingulate region in the physical training group compared to the control group.
Chapman et al. (86)	Physical or Cognitive Training	There were multiple distinct changes on fMRI that suggest that aerobic exercise and cognitive reasoning training contribute differentially to brain health.
Hart et al. (89)	Physical Training	Altered CBF patterns in retired NFL players are concordant with brain regions associated with abnormal findings on neuropsychological testing.
Hotting et al. (91)	Physical and Cognitive Training	Participants of the spatial training group showed lower activity than participants of the perceptual training group in a network of brain regions associated with spatial learning, including the hippocampus and parahippocampal gyrus.
MacIntosh et al. (99)	Physical Training	Perfusion was associated with fitness at baseline and with greater fitness gains with exercise.
McFadden et al. (101)	Physical Training	A six-month exercise intervention was associated with a reduction in default mode network activity in the precuneus.
Xu et al. (109)	Physical Training	Women who engaged in strength training at least once per week exhibited significantly greater cerebrovascular perfusion than women who did not.

CBF= Cerebral Blood Flow, fMRI = functional Magnetic Resonance Imaging, NFL = National Football League.

Smoking

Table 9 outlines the key findings of the individual studies reporting on the relationship between smoking and neurodegeneration in midlife as expressed on fMRI. There were three cross-sectional studies identified in this systematic review that assessed the effect of smoking on brain health as expressed on fMRI. Durazzo et al. (87) investigated the chronic effects of smoking on brain perfusion. Smokers showed significantly lower perfusion than non-smokers in multiple brain regions (bilateral medial and lateral orbitofrontal cortices, bilateral inferior parietal lobules, bilateral superior temporal gyri, left posterior cingulate, right isthmus of cingulate, and right

supramarginal gyrus) as assessed on fMRI. Additionally, greater lifetime duration of smoking (adjusted for age) was related to lower perfusion in multiple brain regions. Gazdzinski et al. (88) assessed one week abstinent alcohol dependent individuals in treatment (19 smokers and 10 non-smokers) and 19 healthy light drinking non-smoking control participants, to assess the concurrent effects of chronic alcohol and chronic smoking on cerebral perfusion. This study found that chronic cigarette smoking adversely affects cerebral perfusion in frontal and parietal grey matter of one-week abstinent alcohol dependent individuals. Mon et al. (114) evaluated cortical grey matter perfusion changes in short-term abstinent alcohol dependent individuals in treatment and assessed the impact of chronic cigarette smoking on perfusion changes during abstinence. At one week of abstinence, frontal and parietal grey matter perfusion in smoking alcoholics was not significantly different from that in non-smoking alcoholics. After five weeks of abstinence, perfusion of frontal and parietal grey matter in non-smoking alcoholics was significantly higher than that at baseline. However, in smoking alcoholics, perfusion was not significantly different. Cigarette smoking appears to hinder perfusion improvement in abstinent alcohol dependent individuals.

Table 9: Key findings of the individual studies reporting on the relationship between lifestyle factors and neurodegeneration in midlife as expressed on fMRI.

Study	Lifestyle Factor	Key findings
Durazzo et al. (87)	Smoking	Smokers showed significantly lower perfusion than non-smokers in multiple brain regions including regions implicated in early Alzheimer's dementia (cingulate, right isthmus of the cingulate, right supramarginal gyrus, and bilateral inferior parietal lobule).
Gazdzinski et al. (88)	Alcohol and Smoking	Alcoholics, as a group, showed lower frontal grey matter perfusion and lower parietal grey matter perfusion than non-smoking light drinkers. In smoking alcoholics, a higher number of cigarettes smoked per day was associated with lower perfusion.
Mon et al. (103)	Smoking	After five weeks of abstinence, perfusion of frontal and parietal grey matter in non-smoking alcoholics was significantly higher than that at baseline. The total number of cigarettes smoked per day was negatively correlated with frontal grey matter perfusion.

Substance misuse

Table 10 outlines the key findings of the individual studies reporting on the relationship between substance use and neurodegeneration in midlife as expressed on fMRI. Of the 12 cross-sectional studies assessing the relationship between substance misuse and neurodegeneration, nine of the studies focused on the effect of cocaine, one on heroin, one on methamphetamine and one on polysubstance misuse.

There were five task-based fMRI studies that looked at the effect of cocaine on brain health. Bednarski et al. (81) used a stop signal task to demonstrate differences in performance between cocaine dependent individuals and healthy controls. Castelluccio et al. (83) assessed individuals with a Go/No-Go task to show differences in BOLD response between cocaine users, former cocaine users and healthy controls. Ide et al. (93) used

power spectrum scale invariance (PSSI) of BOLD signals as a marker of cerebral activity to show disrupted connectivity dynamics in the fronto-parietal region of cocaine addicts. Lee et al (97) evaluated BOLD response to photic visual stimulation to demonstrate evidence of cerebral dysfunction in chronic cocaine abusers. Mitchell et al. (102) used a Stroop task to demonstrate reduced intrinsic connectivity in cocaine dependent individuals compared to healthy controls. Of the four resting-state fMRI studies assessing the effect of cocaine on brain health, both Hu et al. (92) and Kelly et al. (95) assessed resting-state functional connectivity (rsFC). Hu et al. (92) found cocaine addiction is associated with disturbed rsFC in striatal-cortical circuits. Kelly et al. (95) observed reduced prefrontal interhemispheric rsFC in cocaine-dependent participants relative to control subjects. Liu et al. (98) used cerebral oxygen metabolism (CMRO2) as a marker of neural activity and found significantly lower levels in cocaine addicted subjects compared to healthy controls. In a multimodal MRI study, Wang et al. (107) identified hypoperfusion in the prefrontal cortex, anterior cingulate cortex (ACC), insula, right temporal cortex and dorsolateral prefrontal cortex in cocaine addicts compared to controls.

Other than the nine studies focusing on cocaine and neurodegeneration, there were three more cross-sectional studies (one on heroin, one on methamphetamine and one on polysubstance misuse) identified in the systematic review focusing on substance misuse. Jiang et al. (94) investigated amplitude low frequency fluctuate (ALFF) abnormalities in heroin users. Comparing healthy controls and heroin addicts, this resting-

state fMRI study found differing spontaneous neural activity patterns in multiple regions, in the heroin addict group. Heroin addicts had decreased ALFF in the bilateral dorsal anterior cingulate cortex (dACC), bilateral medial orbit frontal cortex, left dorsal lateral prefrontal cortex, left middle temporal gyrus, left inferior temporal gyrus, posterior cingulate cortex and left cuneus as well as increased ALFF in the bilateral angular gyrus, bilateral precuneus, bilateral supramarginal gyrus, left post cingulate cortex and left middle frontal gyrus. Using a task-based fMRI protocol, May et al. (100) found that methamphetamine dependent individuals exhibited altered responses to mechano-receptive C fiber stimulation in brain regions important for interoception. Murray et al (115) assessed brain perfusion in polysubstance users. They found that the combination of cigarette smoking and polysubstance use is strongly related to hypoperfusion in cortical and subcortical regions.

Table 10: Key findings of the individual studies reporting on the relationship between lifestyle factors and neurodegeneration in midlife as expressed on fMRI.

Study	Lifestyle Factor	Key findings
Castelluccio et al. (83)	Substance Misuse	The cocaine user groups exhibited significantly increased BOLD activity relative to healthy controls in several a priori regions of interest.
Hu et al. (92)	Substance Misuse	Compared with healthy controls, the cocaine user group showed differing patterns of rsFC in multiple brain regions.
Ide et al. (93)	Substance Misuse	Compared to healthy controls, cocaine dependent individuals showed decreased PSS and PSSI in multiple frontoparietal regions.
Jiang et al. (94)	Substance Misuse	Compared with controls, heroin addicts had altered ALFF in multiple brain regions.
Kelly et al. (95)	Substance Misuse	Reduced prefrontal interhemispheric rsFC in cocaine dependent participants relative to control subjects and a cocaine dependence related reduction in interhemispheric RSFC among nodes of the dorsal attention network.
Lee et al. (97)	Substance Misuse	Chronic cocaine abusers showed significantly enhanced positive BOLD response to photic stimulation when compared to control subjects.
Liu et al. (98)	Substance Misuse	In cocaine addicted subjects, cerebral metabolic rate of oxygen, a surrogate marker of aggregated neural activity, was significantly lower than that in controls.
May et al. (100)	Substance Misuse	Methamphetamine dependent individuals exhibited lower anterior insula, dorsal striatum, and thalamus activation than healthy controls, across anticipatory and stimulus-related processing.
Mitchell et al. (102)	Substance Misuse	Cocaine dependent patients displayed less overall intrinsic connectivity compared with healthy controls.
Murray et al. (104)	Substance Misuse	Regional perfusion was significantly lower in alcoholics compared to light or non-drinkers. Greater smoking severity correlated with lower perfusion in alcohol dependent individuals.
Wang et al. (107)	Substance Misuse	Compared with controls, cocaine addicted participants showed hypoperfusion and reduced irregularity of resting-state activity in multiple brain regions.

ALFF = Amplitude of Low-Frequency Fluctuation, ANOVA = Analysis of the Variance, BOLD = Blood Oxygenation Level Dependent, CBF = Cerebral Blood Flow, MANOVA = Multivariate analyses of variance, PSS = Post Signal Slowing, PSSI = Power Spectrum Scale Invariance, rsFC = resting-state Functional Connectivity

2.4 Discussion

2.4.1 Main findings

This systematic review identified seven lifestyle factors - physical training, cognitive training, fasting, substance misuse, alcohol, smoking and excessive internet use – which had been researched adequately for our purposes, assessing their impact on neurodegeneration in midlife as expressed on fMRI.

2.4.2 Comparison with other literature and ongoing projects

In late life, the impact of lifestyle factors on brain health has already been studied in multimodal non-pharmacological interventional studies like the Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER) (45). The findings from this double-blind randomised control trial suggest that a 2-year multi-domain intervention that included agreed lifestyle factors (diet, exercise and cognitive training) could maintain or even improve cognitive functioning in an elderly population. In midlife, gathering evidence on the interplay between lifestyle and a broad range of markers of brain health is ongoing in projects like the European Prevention of Alzheimer's Dementia (EPAD) Longitudinal Cohort Study (LCS) (116). The study is aiming to develop a well phenotyped probability spectrum population for Alzheimer's dementia. More specifically data on lifestyle factors and neurodegeneration in midlife as expressed on fMRI is currently being collected in the PREVENT Dementia cohort (53). This is a prospective cohort study to identify midlife biomarkers of late-onset Alzheimer's dementia. The

EPAD LCS (116) and PREVENT Dementia cohort (53) are yet to publish on the relationship between lifestyle factors and neurodegeneration in midlife.

2.4.3 Strengths and limitations

This systematic review used a robust methodology, including a comprehensive search strategy with search terms tailored to each database examined in the review. Additionally, all stages of article screening and quality assessment were undertaken independently by two reviewers. Thus, we can be confident that we identified all the extant studies in the published literature. However, the systematic review has a number of limitations: it did not include grey literature and therefore may have missed unpublished studies such as doctoral theses assessing the relationship between lifestyle and neurodegeneration as expressed on fMRI. There was a marked inconsistency in both study design and methodology of the included studies. Study populations with different demographics were assessed, different fMRI protocols were used by the studies (18 task-based and 11 resting-state), different statistical approaches to analyse the data and different fMRI outcome measures all added to the heterogeneity and meant a meta-analysis could not be conducted. Additionally, caution is necessary when interpreting fMRI outcome measures and how they relate to underlying neural activity. More studies are needed to provide a firmer understanding of situations when fMRI outcome measures and neural activity are coupled and when they dissociate (117).

2.4.4 Implications

This systematic review contributes to our endeavour to gain a better understanding of what lifestyle factors could be optimised to help improve brain health in midlife and therein reduce an individual's risk of later life dementia. More specifically, in chapter 3, the information retrieved by this systematic review has guided the analysis of existing lifestyle and ASL data in the PREVENT Dementia cohort (53). Additionally, this systematic review could help shape future multimodal non-pharmacological midlife dementia prevention studies which target lifestyle modification.

2.4.5 Conclusions

From this systematic review, common themes have emerged on the effect of lifestyle on brain health in midlife as expressed on fMRI. The findings are summarised in Table 11, which shows all the cognitive training studies demonstrated what could be considered a neuroprotective effect, whereas all the alcohol, smoking and substance misuse studies demonstrated an effect that could reflect an early phase of neurodegeneration. Studies on physical training showed more variation in results. The majority of physical training studies showed a potential neuroprotective effect and one study showed no effect. One study described a possible neurodegenerative effect associated with physical training; in this study by Hart et al. (89), the majority of the ex-NFL players assessed, had a history of concussion. The evidence from studies on the effects of excessive internet use and fasting on brain health in midlife is too limited to allow any conclusions to be drawn.

Table 11: Interpretation of the findings from the individual studies reporting on the relationship between lifestyle factors and neurodegeneration in midlife as expressed on fMRI.

Lifestyle Factor	**Interpretation of the findings from the individual studies**	**Studies**
Alcohol	Neurodegeneration	Gazdzinski et al. (88), Hermann et al. (90), Kim et al. (96), Rogers et al. (105), Sullivan et al. (106) and Weiland et al. (108).
Cognitive Training	Neuroprotection	Chapman et al. (85), Chapman et al. (86) and Hotting et al. (91).
Excessive internet use	Neurodegeneration	Kim et al. (96)
Fasting	Neuroprotection	Belaich et al. (82)
Physical Training	Neuroprotection	Chapman et al. (84), Chapman et al. (86), MacIntosh et al. (99), McFadden et al. (101) and Xu et al. (109).
Physical Training	No effect	Hotting et al. (91)
Physical Training	Neurodegeneration	Hart et al. (89)
Smoking	Neurodegeneration	Durazzo et al. (87), Gazdzinski et al. (88), Mon et al. (103)
Substance Misuse	Neurodegeneration	Bednarski et al. (81), Castelluccio et al. (83), Hu et al. (92), Ide et al. (93), Jiang et al. (94), Kelly et al. (95), Lee et al. (97), Liu et al. (98), May et al. (100), Mitchell et al. (102), Murray et al. (104) and Wang et al. (107).

2.4.6 Implications of results

The findings of the systematic review will be used to guide the analysis of the PREVENT Dementia cohort baseline data and could be used as the basis for the future analysis of the yet to be released two year follow-up data from the PREVENT Dementia cohort (53). However it is worth noting that the systematic review it based only on published studies. Therefore the results and conclusions drawn from the systematic review are significantly influenced by publication bias. Of note, there is evidence to suggest this bias is increasing. An investigation covering more than 4600 publications from

different countries and disciplines found strong evidence for a steady and significant increase in publication bias over the years. The frequency of papers declaring significant statistical support for their a priori formulated hypotheses increased by 22% between 1990 and 2007 (n = 4656, p < 0.001) (118).

3 Analysis of the relationship between lifestyle and arterial spin labelling cerebral blood flow in midlife using data from the PREVENT Dementia cohort

Chapter abstract

INTRODUCTION: The findings from the systematic review (chapter 2) were used to guide the linear regression analysis exploring the relationship between lifestyle factors and neurodegeneration in midlife as expressed on arterial spin labelling using data from the PREVENT Dementia cohort.

METHODS: Linear regression models were used to evaluate the relationship between lifestyle factors (physical training, alcohol, smoking and substance misuse) and neurodegeneration in midlife as expressed on arterial spin labelling. Regional cerebral blood flow was used as an outcome measure.

RESULTS: Across the unadjusted and adjusted linear regression models, compared to being a non-substance misuser, being a current or ex-substance misuser was associated with a reduction in anterior cingulate cortex cerebral blood flow. Additionally, adjusted model 2 showed that compared to being a moderate alcohol drinker, being a non-drinker was associated with a decreased anterior cingulate cortex cerebral blood flow.

DISCUSSION: The analysis of the data from the PREVENT Dementia cohort has added to the evidence that lifestyle factors are associated with cerebral blood flow in the anterior cingulate cortex. It may well be that the anterior cingulate is a sensitive marker for the effect of lifestyle on neurodegeneration in midlife as expressed on arterial spin labelling.

3.1 Background

In the systematic review of the relationship between lifestyle and neurodegeneration in midlife as expressed on fMRI (chapter 2) (72), seven lifestyle factors (physical training, cognitive training, fasting, substance misuse, alcohol, smoking and excessive internet use) associated with neurodegeneration in midlife as expressed on fMRI were identified. Physical training and cognitive training have a likely neuroprotective effect. Substance misuse, alcohol and smoking have a likely neurodegenerative effect. Further studies are needed to clarify the effects of fasting and excessive internet use on fMRI outcomes in midlife. The findings of this systematic review have been brought forward to analyse the impact of lifestyle on cerebral blood flow (CBF) in a midlife cohort using the data from the PREVENT Dementia cohort (53). The following hypothesis was tested: Physical training, cognitive training, substance misuse, alcohol and smoking in midlife are associated with regional cerebral blood flow as expressed on arterial spin labelling (ASL).

3.2 Method

3.2.1 The Prevent Dementia cohort

Aims and Objectives

Baseline assessment data from a single site, the Imperial College London recruitment site for the PREVENT Dementia cohort (119) were used to assess the relationship between lifestyle and neurodegeneration in midlife as expressed on ASL. The data were for individuals in midlife, aged 40-59 years

old at baseline. Recruitment for the PREVENT Dementia cohort (119) is now ongoing across five research centres - Edinburgh, Dublin, London, Oxford and Cambridge in the United Kingdom. However at the time of undertaking my thesis, data were only available from the original research centre at Imperial College London.

The primary aim of the PREVENT Dementia cohort (119) is understanding the interplay between risk factors for neurodegeneration, identifying markers for the biological expression of neurodegenerative disease, developing surrogate markers for measuring future reductions in dementia incidence and developing secondary prevention intervention studies in midlife. Ethical approval was obtained by the St Giles and Camberwell (London) Ethics Committee.

Recruitment into the PREVENT Dementia cohort

Participants were recruited to the PREVENT Dementia cohort (119) baseline assessment at the Imperial College London recruitment site from a variety of different sources: NHS clinical appointments; the Join Dementia Research website (https://www.joindementiaresearch.nihr.ac.uk/); marketing materials such as leaflets about the study; or social media such as the PREVENT Dementia cohort (119) website (http://preventdementia.co.uk/). Additionally, potential participants were drawn from the electronic patient record system in the West London Mental Health NHS trust and asked whether they would like to participate in the programme. Alternatively, with the approval of a GP practice, a GP database was accessed to search for

potential participants. Between September and December 2019, I had ongoing contact with the Imperial College London recruitment site to clarify how many people were approached to take part in the study and how many individuals were recruited by each of the methods outlined above. Unfortunately the Imperial College London recruitment site were unable to provide the requested information before the publication of this thesis.

Following initial contact, information about the PREVENT Dementia cohort (119) was discussed and screening for eligibility took place either face to face or on the telephone. If the participant was still eligible and interested, study related information was given to them. This included participant information sheets, a consent form and the MRI eligibility checklist. Once the participant returned their consent form to the research unit, the participant was contacted by the study coordinator who then scheduled an appropriate time slot for the participant to attend the research unit. The MRI scan was completed within one month of the clinical visit.

Inclusion and Exclusion Criteria

The inclusion and exclusion criteria for the PREVENT Dementia cohort (119) are as follows;

Inclusion

1. Aged 40-59 years old at time of consent.
2. Participant fluent in English.

Exclusion

1. Known diagnosis or report of subjective cognitive impairment or dementia using standard clinical criteria (DMS IV(120), ICD 10 (121)).

2. Participant has a known contraindication to MRI.

Clinical Assessment

Participants were consented according to the International Council on Harmonisation (ICH) good clinical practice (GCP) guidelines (122). Data were collected using a range of assessments administered by a member of the PREVENT Dementia cohort (119) team. The following sources were reviewed for demographic and lifestyle data.

i. PREVENT case report form – This is a comprehensive protocol driven document to collect participant data and includes a physical examination of the patient.
ii. Simpson Angus Scale (123) – This is a rating scale to assess for extrapyramidal disturbance.
iii. Apathy inventory (124) – This is a rating scale for global assessment of apathy and separate assessment of emotional blunting, lack of initiative, and lack of interest.
iv. Lawton instrumental activities of daily life (IADL) scale (125) – This rating scale assesses how a person is functioning at the present time and can be used to monitor for improvement or deterioration over time.
v. National Adult Reading Test (NART) (126) – This test is a widely accepted and commonly used method for estimating premorbid intelligence of English speaking participants in neuropsychological research.

vi. Addenbrooke's Cognitive Examination 3rd revision (ACE III) (127) – This is a screening test to assess five cognitive domains; attention, memory, verbal fluency, language and visuospatial abilities.

vii. Supermarket task (128) - This is a test of spatial orientation and memory. It is completed by the participant using the iPad tablet. The task requires the participant to follow a route through a virtual supermarket from behind a trolley and involves a series of 90° turns. The subject is then required to point in the direction of the supermarket entrance.

viii. Four Mountains task (129) - This is a test of spatial orientation and memory. The Four Mountains task is also completed on the iPad. The test assesses memory for places, by showing a picture of a landscape that contains four mountains and testing people's ability to identify the same landscape when seen from a different angle.

Additionally, the participant completed the following self-report questionnaires:

i. Pregnancy and Menstruation – this questionnaire collects data related to women's menstrual cycle, history of pregnancy and use of hormone replacement therapy.

ii. Lifetime of Experiences Questionnaire (LEQ) (130) - this questionnaire assesses a range of complex mental pursuits and physical activity during middle age years. In the LEQ, middle age years is defined as after the age of 30 years and until the end of working life / present.

iii. Centre for Epidemiologic Studies Depression Scale (CES-D) (131) – This is a short self-report scale designed to measure depressive symptomatology in the general population.

iv. Spielberger state scale (132)- This is a self-report scale designed to measure anxiety.

v. Pittsburgh Sleep Quality Index (PSQI) (133) – This self-rated questionnaire assesses sleep quality and disturbance over a one month time period.

vi. Connor-Davidson Resilience scale (134) – This is a 25 item scale to measure an individual's ability to cope with stress.

vii. Life Stressor Checklist – Revised (135) – This self-rated questionnaire assesses traumatic or stressful life events. The measure has a focus on events relevant to women such as abortion, although it can also be used with men. The questionnaire includes 30 life events, including experiences with natural disasters, physical or sexual assault and the death of a relative.

viii. Brain Injury Screening Questionnaire (BISQ) (136) - This self-rated questionnaire is a screening tool used to document lifetime history of self-reported traumatic brain injury and the presence of current symptoms.

ix. Food Frequency Questionnaire (FFQ) (137) – This is a semi quantitative questionnaire designed to estimate daily intake of a wide range of nutrients.

x. Visual Short term memory binding test (138)- Short-term memory binding is a memory function that underpins the temporary retention of complex objects e.g. shapes with colours. This test assesses the recognition of shapes, colours or shape-colour bindings.

xi. COGNITO: Computerized Assessment of Information Processing (139) – This neuropsychological battery primarily assesses attentional, linguistic, amnesic and visuospatial processes.

Figure 7 shows the sources that lifestyle and demographic data were extracted from. It shows that demographic, alcohol, smoking and substance use data were extracted from the Prevent case report form. Additionally, physical training data were extracted from the LEQ.

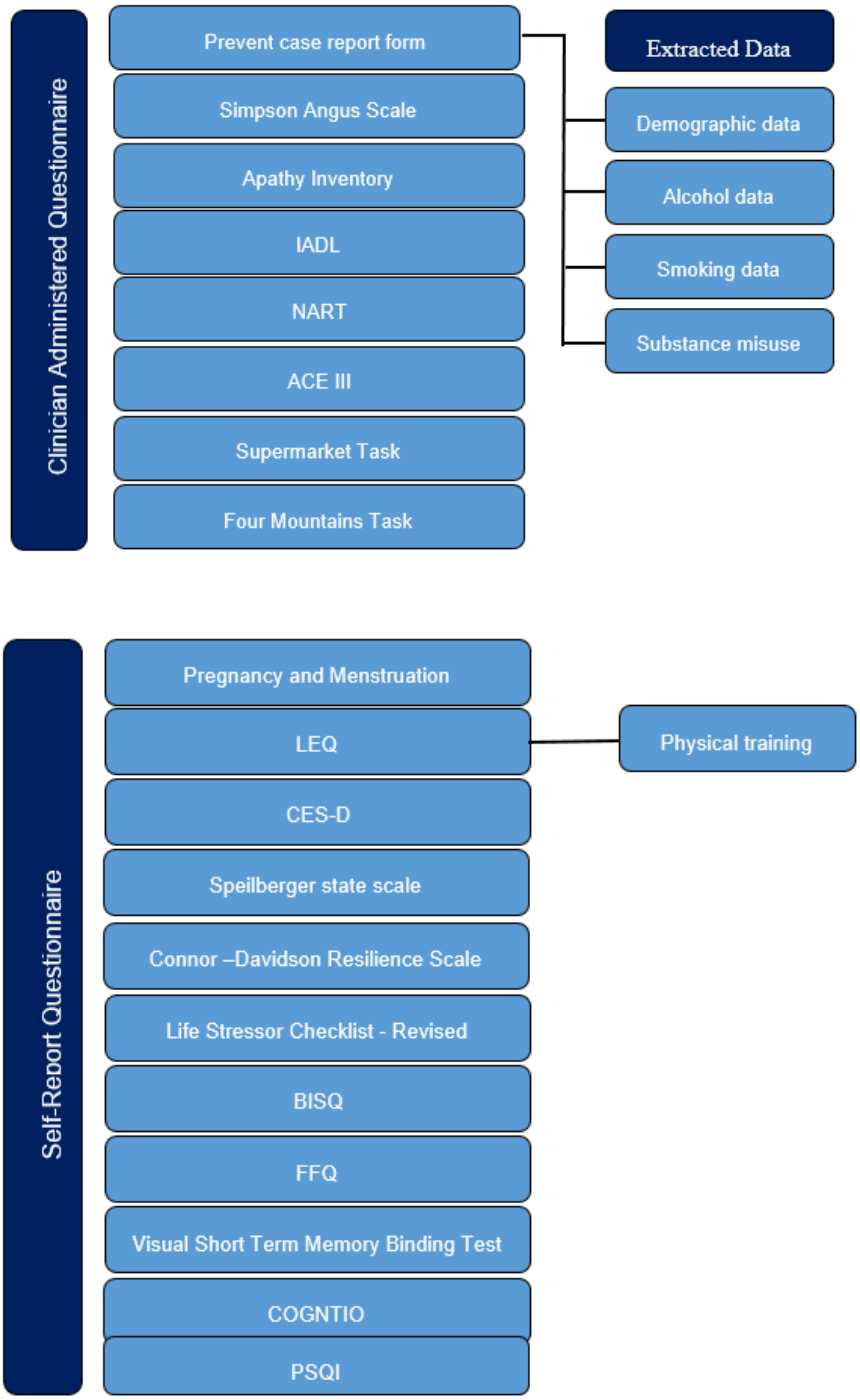

Figure 7: Data sources for demographic and lifestyle data.

In addition to completing questionnaires, a clinical assessment was made of each person's physical and mental health. This confirmed that they do not have dementia and that they were fit to participate in the longitudinal cohort study. Furthermore, saliva, blood and urine samples were collected to assess for biomarkers of neurodegeneration in midlife and *APOE* genotyping.

MRI acquisition

As part of the assessment for the PREVENT Dementia cohort (119), all participants underwent an 80 minute MRI scan.

MRI protocol

i. Whole brain volumetric T1 and T2 weighted images for segmentation and volumetric analyses.
ii. Fluid attenuation inversion recovery (FLAIR) sequences to allow identification and quantification of deep white matter hyperintensities.
iii. Diffusion weighted imaging (DWI) for identification of changes in normal appearing white matter and major tracts.
iv. Susceptibility-weighted imaging (SWI) for identification of microbleeds.
v. Magnetic Resonance Spectroscopy (MRS) for assessment of early metabolic changes.
vi. FMRI; both task-based and resting-state, including ASL for assessment of CBF.

PREVENT fMRI Task

The PREVENT fMRI task is an encoding and retrieval task. In the encoding stage, images of indoor and outdoor scenes are displayed on a screen for the participant to view whilst the participant is in the MRI scanner. The task is to judge if the image represents an ‘indoor’ or ‘outdoor’ scene. After each image, a question mark is seen in the centre of the screen, in addition to buttons in the left and right-hand corner of the screen denoting the ‘indoor’ and ‘outdoor’ answer options. The button in the participant’s left hand corresponds to the button on the left-hand side of the screen and the button in the participant’s right hand corresponds to the button on the right-hand side of the screen. The participant makes a response as quickly as possible, before the next image appears.

In the retrieval stage, the task is now to indicate whether or not the image on the screen was previously seen in the encoding task. If the participant thinks the image was in the previous indoor/outdoor task, the button is pressed that corresponds to “old”. If the participant does not think they saw the scene in the previous task, the button is pressed that corresponds to "new".

Imaging Data Analysis

The imaging data from the PREVENT Dementia cohort (119) were acquired at Imperial College London and transferred to Cambridge University for processing. All the imaging data analysis was undertaken by the Department of Neuroscience, Cambridge University using the Freesurfer

image analysis suite (www.surfer.nmr.mgh.harvard.edu/). Processing included motion correction and averaging (140) of multiple volumetric T1 weighted images, removal of non-brain tissue using a hybrid watershed and surface deformation procedure (141) and automated Talairach transformation. Segmentation of the subcortical white matter and deep grey matter volumetric structures (142), intensity normalization (143) and tessellation of the grey and white matter boundary were undertaken. Finally automated topology correction (144) and surface deformation following intensity gradients to optimally place the grey, white and cerebrospinal fluid borders were completed (145).

Individual CBF maps were co-registered to the processed individual T1 images using a boundary-based registration algorithm (146). Volume to surface normalisation and smoothing were undertaken using FreeSurfer (147). Regions of interest (ROI) were defined according to the Desikan-Killiany atlas (148). For each participant, mean CBF values for each region of interest were extracted.

Data access and extraction

The systematic review of the relationship between lifestyle and neurodegeneration in midlife as expressed on fMRI, identified five lifestyle factors associated with neurodegeneration in midlife – physical training, cognitive training, alcohol, smoking and substance misuse. Therefore the available data from the PREVENT Dementia cohort (119) on these five lifestyle factors were extracted. The PREVENT Dementia cohort (119)

interview questions on physical training, alcohol, smoking and substance misuse are outlined in Table 12, 13, 14 and 15. The tables show that in the Lifetime of Experiences Questionnaire, data had been collected on the frequency of mild, moderate and vigorous physical activity. In the Prevent case report form, data had been collected on medical conditions related to alcohol and alcohol intake. Additionally, data were collected on smoking, medical conditions related to substance misuse and the type of substances that an individual had used. There were no data on cognitive training available.

Table 12: **PREVENT Dementia cohort physical training data.**

Physical training questions	Source
Question: How often would you take part in sports or activities that are mildly energetic? E.g. walking, woodwork, weeding, hoeing, bicycle, repair, playing pool, general house work. Responses: Never | Less than monthly | Monthly | Fortnightly | Weekly | Daily.	LEQ (130)
Question: How often would you take part in sports or activities that are moderately energetic? E.g. scrubbing, polishing car, dancing, golf, cycling, decorating, lawn mowing, leisurely swimming. Responses: Never | Less than monthly | Monthly | Fortnightly | Weekly | Daily.	LEQ (130)
Question: How often would you take part in sports or activities that are vigorous? E.g. running, hard swimming, tennis, squash, digging, cycle racing. Responses: Never | Less than monthly | Monthly | Fortnightly | Weekly | Daily.	LEQ (130)

Table 13: **PREVENT Dementia cohort alcohol data.**

Alcohol questions	Source
Question: Alcoholism - has had condition? Response: Yes | No.	PREVENT case report form (medical history)
Question: Alcoholism - currently active? Response: Yes | No.	PREVENT case report form (medical history)
Question: Alcoholism - hospitalised for condition? Response: Yes | No.	PREVENT case report form (medical history)
Question: Alcohol intake status? Response: Non-drinker | Ex-drinker | Current drinker | Unknown.	PREVENT case report form (lifestyle interview)
Question: Number of glasses of wine / beer per week? Response: XXX glasses.	PREVENT case report form (lifestyle interview)
Question: Number of glasses of stronger alcohol per week? Response: XXX glasses.	PREVENT case report form (lifestyle interview)
Question: Total number of units per week? Response: XXX units.	PREVENT case report form (lifestyle interview)

Table 14: **PREVENT Dementia cohort smoking data.**

Smoking questions	Source
Question: Smoking status? Response: Non-smoker | Ex–smoker | Current smoker | Unknown.	PREVENT case report form (lifestyle interview)
Question: Number of years smoked? Response: xxx years.	PREVENT case report form (lifestyle interview)
Question: Number of cigarettes / cigars per day? Response xxx cigarettes / cigars	PREVENT case report form (lifestyle interview)

Table 15: **PREVENT Dementia cohort substance misuse data.**

Substance misuse questions	Source
Question: Substance abuse - has had condition? Response: Yes | No.	PREVENT case report form (medical history)
Question: Substance abuse - currently active? Response: Yes | No.	PREVENT case report form (medical history)
Question: Substance abuse - hospitalised for condition? Response: Yes | No.	PREVENT case report form (medical history)
Question: Substance misuse status? Response: Never | Ex–user | Current user | Unknown.	PREVENT case report form (lifestyle interview)
Question: Type of drug used? Response: Sedatives-hypnotics-anxiolytics |Cannabis | Stimulants | Opioids |Cocaine | Hallucinogens | Other.	PREVENT case report form (lifestyle interview)

3.2.2 Statistical Analysis

Statistical analysis plan

The aim of the statistical analysis was to test the following hypothesis: Physical training, cognitive training, substance misuse, alcohol and smoking in midlife are associated with regional cerebral blood flow as expressed on ASL. R Studio Version 1.0.143 was used to carry out the statistical analysis. In accordance with guidance by Goldacre (149), to enhance transparency and reproducibility, all analytic code used in this analysis has been made available. Please see Appendix E for the analytic code used in this analysis. As missing data were limited to a small number of observations, individuals with missing ASL data were excluded from analysis by listwise deletion.

Univariate and multivariate linear regression were undertaken to analyse the relationship between lifestyle factors and ASL CBF. In keeping with convention, the reference group for a lifestyle factor was the group with

the largest number of individuals. For example, the reference group for physical training was the high physical training score (N=114) group. Therefore the medium physical training score group (N=84) and low physical training score group (N=12) were compared against the high physical training score group in the linear regression models. The dependent variable was CBF and the independent variables were known risk factors for Alzheimer's dementia and lifestyle factors. For multiple regression analyses, the *p*-value was set at 0.05. Effect size is an objective and standardised measure of the magnitude of observed effect (150). Pearson's correlation coefficient (151) was used to measure effect size.

Covariates identified from the systematic review

In the systematic review, 29 studies were identified that assessed the relationship between lifestyle and neurodegeneration in midlife as expressed on fMRI. Of these 29 studies, there were 11 studies with a global quality rating of strong based on the Effective Public Health Practice Project Quality Assessment Tool (EPHPP) (77). Nine of these studies assessed lifestyle factors identified in the PREVENT Dementia cohort (119). All nine studies adjusted for age, five studies adjusted for gender, six studies adjusted for education, four studies for IQ and two for cognition. Additionally, five studies also adjusted for other factors including body mass index (BMI) and ethnicity. The factors adjusted for in the nine studies with an EPHPP rating of strong are shown in Table 16 and 17. Based on these findings - age, gender and

education were chosen as covariates in the analysis of the relationship between lifestyle and neurodegeneration in midlife as expressed on ASL.

Table 16: Key factors adjusted for in statistical analysis of the nine strong quality studies identified in the systematic review of the relationship between lifestyle and neurodegeneration in midlife as expressed on fMRI.

Study	Lifestyle Factor	Age	Gender	Education	IQ	Cognition	Other
Chapman et al. (86)	Cognitive training & physical training	✓	✓	×	✓	✓	×
Chapman et al. (113)	Physical training	✓	✓	×	✓	✓	×
Hart et al. (89)	Physical training	✓	×	✓	✓	×	×
Xu et al. (109)	Physical training	✓	✓	×	×	×	✓
Mon et al. (103)	Alcohol & smoking	✓	×	✓	×	×	×
Jiang et al. (94)	Substance misuse	✓	×	✓	×	×	✓
Kelly et al. (95)	Substance misuse	✓	✓	✓	×	×	✓
Mitchell et al. (102)	Substance misuse	✓	✓	✓	✓	×	✓
Murray et al. (115)	Substance misuse	✓	×	✓	×	×	✓
Total		**9**	**5**	**6**	**4**	**2**	**5**

Table 17: Other factors adjusted for in statistical analysis of the 11 strong quality studies identified in the systematic review of the relationship between lifestyle and neurodegeneration in midlife as expressed on fMRI.

Study	Lifestyle factor	Other factors adjusted for in statistical analysis
Xu et al. (109)	Physical training	Body mass index (BMI), cardiovascular disease (CVD) status, cardiovascular medications, cardiac index, physical limitations, self-reported physical health, participation in aerobic activity, flexibility training and whole brain volume.
Jiang et al. (94)	Substance misuse	Head motion and cigarette smoking.
Kelly et al. (95)	Substance misuse	Employment status.
Mitchell et al. (102)	Substance misuse	Ethnicity.
Murray et al. (115)	Substance misuse	Body mass index (BMI), American National Adult Reading Test (AMNART) score and drinking variables.

Covariates based on established non-modifiable risk factors for Alzheimer's dementia

Apolipoprotein E (*APOE*) allele and family history alter an individual's risk of Alzheimer's dementia (152). The *APOE* gene that lies on chromosome 19 has a major impact on Alzheimer's dementia risk (59). The *APOE* gene has three alleles, the *APOE* ε4 allele increases risk and *APOE* ε2 allele decreases risk. The *APOE* ε3 allele is associated with average risk (153). Family history of dementia is an important risk factor for Alzheimer's dementia independently of known genetic risk factors for Alzheimer's dementia (154). Given that early Alzheimer's dementia may well present as neurodegeneration in midlife (70), data on the number of parents with dementia and *APOE* status were included in the data analyses.

Conceptualization of lifestyle factors

The decision on how best to conceptualise the lifestyle data from the PREVENT Dementia cohort (119) were based on the findings from the systematic review (chapter 2) and is outlined below.

Physical Training

In the systematic review, different physical training programmes were used by different studies. Studies by Chapman et al. (86, 113) used a three month aerobic training programme, whilst Hotting et al. (91) used a six month aerobic training programme. Participants in a study by MacIntosh et al (99) undertook a six month aerobic and resistance training programme and participants in a study by McFadden et al. (101) adhered to a six month treadmill walking protocol. Hart et al. (89) assessed brain health in retired professional National Football League players and in a cross-sectional study, Xu et al. (109) measured levels of physical training in the general population using the Rapid Assessment of Physical Activity (RAPA) questionnaire (155). The RAPA questionnaire (155) is an evidence based tool that assesses physical activity. The RAPA questionnaire has two sections, RAPA 1 and RAPA 2. RAPA 1 takes into account the frequency and intensity of physical training to give a single summative score. RAPA 2 assesses whether strength training and flexibility training are undertaken. Based on RAPA 1, the physical training data from the PREVENT Dementia cohort (119) has been transformed into a single summative score taking into account the frequency and intensity of physical training.

The scoring system for the PREVENT physical training score is outlined in Table 18. The PREVENT physical training score is based on the frequencies that three different intensities of training - mildly energetic training, moderately energetic training and vigorously energetic training are undertaken. Depending on the frequency of training, a score ranging from 0 to 5 can be obtained for each intensity of training. These three scores were then combined to give a physical training score. A score of 0 to 5 would suggest a low level of physical fitness, 6 to 10 would suggest a moderate level of physical fitness and a score of 11 to 15 would suggest a high level of physical fitness.

Table 18: Physical training score calculation.

	Never	Less than monthly	Monthly	Fortnightly	Weekly	Daily	Total
Mildly energetic	0	1	2	3	4	5	0-5
Moderately energetic	0	1	2	3	4	5	0-5
Vigorously energetic	0	1	2	3	4	5	0-5
Physical training score (mildly energetic score + moderately energetic score + vigorously energetic score).							0-15

Alcohol

In the systematic review of the relationship between lifestyle and neurodegeneration in midlife as expressed on fMRI, seven studies assessed the effect of alcohol. Of the seven studies, six assessed alcohol drinkers who had a diagnosis of alcohol abuse or alcohol dependence as defined by DSM-IV (156) or ICD-10 (121). In one study, Weiland et al. (108) assessed individuals who partook in alcohol binge drinking. In the PREVENT Dementia cohort (119), data is collected on alcohol dependence, although the frequency of this diagnosis is likely to be low as participants for the study are recruited from the general population. Data is not collected on the frequency of alcohol binge drinking in the PREVENT Dementia cohort (119). Therefore, for the purposes of data analysis, the PREVENT alcohol data has been split into two groups; non-drinkers and current or ex-drinkers. The current or ex-drinkers group has been further divided, based on whether individuals drink within the recommended limits as outlined in the new alcohol guidelines launched by the Department of Health (157) in the United Kingdom on 8th January 2016. PREVENT Dementia cohort (119) participants that drink less than 14 units per week have been defined as moderate alcohol drinkers and participants who drink more than 14 units per week have been defined as heavy alcohol drinkers.

Smoking

In the systematic review of the relationship between lifestyle and neurodegeneration in midlife as expressed on fMRI, three studies looked at

the relationship between smoking and neurodegeneration. Durazzo et al. (87) defined smokers as individuals who were actively smoking at the time of assessment and who have smoked at least ten cigarettes per day for at least five years. Gazdzinski et al. (88) and Mon et al. (103) both divided participants in their studies into two groups; non-smokers and smokers.

For the purposes of further analysis, participants in the PREVENT Dementia cohort (119) have also been divided into two groups; non-smokers and current or ex-smokers.

Substance misuse

There were twelve studies identified by the systematic review that looked at the relationship between substance misuse and neurodegeneration in midlife as expressed on fMRI. All the studies looked at individuals who met the DSM-IV (156) criteria for substance dependence. Nine of the studies focused on the effect of cocaine, one on methamphetamine, one on opiates and one on the effect of polysubstance misuse on brain health in midlife.

In the PREVENT case report form, data is collected on whether participants have been diagnosed with a substance abuse disorder, whether they misuse substances and the type of substances that they misuse. In the PREVENT Dementia cohort (119), the frequency of a diagnosis of substance misuse is likely to be low, as participants for the study are recruited from the general population. For data analysis, participants in the PREVENT Dementia cohort (119) were broadly divided into two groups, those that have

never misused substances in the past and participants who are current or ex-substance misusers.

MRI data

MRI measures were received from the PREVENT Dementia cohort (119) as analysed imaging data became available from Cambridge University. The following MRI measures were available for analysis:

i. Cerebral microbleeds present (yes / no).
ii. Total number of cerebral microbleeds.
iii. Region of cerebral microbleed.
iv. Total white matter volume hyperintensity percentage.
v. Regional white matter volume hyperintensity percentage.
vi. Periventricular hyperintensities (PVH) Fazekas score (158).
vii. Deep white matter hyperintensities (DWMH) Fazekas score (158).
viii. Total brain volume.
ix. Grey matter volume (GMV).
x. White matter volume (WMV).
xi. CSF volume.
xii. Hippocampal grey matter volume.
xiii. Parahippocampal grey matter volume.
xiv. Hippocampus mean CBF.
xv. Anterior cingulate cortex (ACC) mean CBF.
xvi. Mean cortex CBF.

Arterial Spin Labelling Cerebral Blood Flow

Arterial Spin Labelling (ASL) Cerebral Blood Flow (CBF) refers to the rate of delivery of arterial blood to the capillary bed in brain tissue and is typically quantified in millilitres of blood per 100 grams of tissue per minute (159). In ASL, arterial blood water is magnetically labelled just below the region of interest. After a period of time to allow the blood to travel to the tissue (post-labelling delay), the magnetically labelled arterial blood water flows into slice of interest where it exchanges with tissue water. During this time, an image is taken (tag image). The experiment is then repeated without labelling the arterial blood to create another image (control image). The control image and the tag image are subtracted from each other to produce a perfusion image. This image will reflect the amount of arterial blood delivered to each voxel in the tissue slice within the transit time (160).

In my thesis, the three ASL regions of interest chosen for analysis were hippocampus CBF, ACC CBF and cortex CBF. These regions were chosen based on three factors. Firstly, the findings of the systematic review of the relationship between lifestyle and neurodegeneration as expressed on fMRI. In addition to this, studies showing that ASL CBF is a promising biomarker for neurodegeneration and discussion with the PREVENT Dementia Neuroimaging team.

The systematic review of the relationship between lifestyle and neurodegeneration in midlife as expressed on fMRI identified three studies from the Centre for Brain Health, University of Texas that looked at the

relationship between lifestyle factors and CBF measured using pseudocontinous arterial spin labelling (85, 86, 113). All three studies had an EPHPP quality rating of strong. In the first study, Chapman et al. demonstrated higher resting ACC CBF in a physical training group compared to a control group. The study hypothesised that ACC CBF may be a sensitive marker in older adults of gains in brain health (113). In the second study, Chapman et al. demonstrated that complex cognitive training increases global CBF compared to a control group (85). In a third study, by Chapman et al, looking at the benefits of cognitive training vs physical training, the study found that increased cognitive and physical activity improves brain health in distinct ways. Of note, increased physical activity was associated with increased hippocampus CBF (86).

Studies have shown that ASL CBF is a promising biomarker for neurodegeneration. Compared to age-matched cognitively normal adults, individuals with Alzheimer's dementia show an approximately 40% global decrease in CBF (58). Furthermore, possessing the *APOE* ε4 allele has been shown to increase Alzheimer's disease risk by 3 to 8 fold (59). The effect of *APOE* on CBF appears to be mediated by age, with evidence that older *APOE* ε4 adults display greater hypoperfusion and younger *APOE* ε4 adults show greater hyperperfusion in the ACC (161). The hyperperfusion observed in younger *APOE* ε4 carriers was correlated with better executive functioning, suggesting compensatory mechanisms may be engaged many years prior to symptom onset (161).

The research outlined above was discussed with the PREVENT Dementia Neuroimaging team at Cambridge University before the PREVENT Dementia Cohort ASL CBF data was chosen for further analysis. As a biomarker for neurodegeneration, ASL confers specific benefits, it is non-invasive and it is safe to repeat over time. ASL can therefore be used to track changes in CBF such as those due to disease progression or drug therapy (162). However there are multiple limitations associated with ASL. The specificity of ASL CBF to distinguish between Alzheimer's dementia pathology and other vascular aetiologies has not been firmly established, as reduced CBF has also been reported in vascular dementia and in a post-stroke non-dementia group (163, 164). This cautions against the use of CBF measures in isolation, without considering other information such as neuropsychological performance and clinical assessment (65).

3.3 Results

3.3.1 Participant Characteristics

The baseline assessment data for the Imperial College London recruitment site for the PREVENT Dementia cohort (119) descriptive characteristics are shown in Table 19. Of the 210 participants, the average age of participants was 52 years old. There were 75 (29.5%) participants who had at least one *APOE* ε4 allele and 133 (63.9%) participants had no *APOE* ε4 allele. It is notable that the mean number of years of education was 16, this would suggest a high level of education amongst participants in the PREVENT Dementia cohort (119). There were 62 (29.5%) male and 148

(70.5%) female participants. The participants' characteristics were similar for men and women subgroups and are shown in Table 19.

Of the 210 participants, there were 161 participants with no missing data and ASL outcome data. The participant characteristics were similar for ASL subgroup (N=161) compared to the Prevent Dementia cohort (N=210) (119). For the ASL subgroup, the mean age was 52 years. Of these participants, 44 (27.3%) were male and 117 (72.7%) were female. At least one *APOE* ε4 allele was present for 100 (62.1%) participants and 61(37.9%) had no *APOE* ε4 allele.

In comparison with the ASL subgroup (N=161), the individuals not included in the analysis due to missing data (N=49) were more likely to be male. In the ASL subgroup, 27.3% of the participants were male compared to 36.7% in the data set that was not included in analysis. Furthermore in the ASL subgroup, the mean number of years of education was higher than that in the data set for individuals not included in analysis. In the ASL subgroup, the mean number of years of education was 16 years compared to 15.5 years in the data set not included in analysis.

Table 19: Summary of Prevent Dementia cohort demographic characteristics.

Demographics	N=210 (full data set)	N=62 (males)	N=148 (females)	N=161 (ASL subgroup)	N = 49 (not included in analysis due to missing data)
Age - mean ± SD	52.0 ± 5.5	52.0 ± 5.6	51.9 ± 5.5	51.8 ± 5.5	52.3 ± 5.2
Gender: M / F - N (% of total sample)	62 (29.5) / 148 (70.5)	NA	NA	44 (27.3) / 117 (72.7)	18 (36.7) / 31 (63.3)
APOE ε4 allele: No *APOE* ε4 / *APOE* ε4 ≥ 1 – N (% of total sample)	133 (63.9) / 75 (36.1)	40 (64.5) / 22 (35.5)	93 (63.7) / 53 (36.3)	100 (62.1) / 61 (37.9)	35 (71.4) / 14 (28.6)
Race: Caucasian / other - N (% of total sample)	188 (89.5) / 22 (10.5)	50 (80.6) / 12 (19.4)	138 (93.2) / 10 (6.8)	146 (90.7) / 15 (9.3)	42 (85.7) / 7 (14.3)
Number of years of education: mean + SD	15.9 ± 3.4	15.8 ± 3.5	15.9 ± 3.4	16.0 ± 3.4	15.5 ± 3.4
Domiciliary status: lives with others / lives alone – N (% of total sample)	180 (85.7) / 30 (14.3)	58 (93.5) / 4 (6.5)	122 (82.4) / 26 (17.6)	136 (84.5) / 25 (15.5)	45 (91.8) / 4 (8.2)

APOE = Apolipoprotein, N = Number, SD = Standard deviation, NA=Not applicable

3.3.2 Neuroprotective lifestyle factor

Physical training

The PREVENT Dementia cohort (119) physical training data are summarized in Figure 8. The data shows that 136 participants engage in mildly energetic activity on a daily basis, 36 participants engage in moderately energetic activity on a daily basis and 10 participants engage in vigorously energetic activity on a daily basis. There were 114 participants with a high physical training score, 84 participants with a medium physical training score and 12 participants with a low physical training score. The

physical training scores ranged from 1 to 15 and the mean score is 10.7 (SD 2.9). This indicates the majority of participants had a medium or high level of physical fitness.

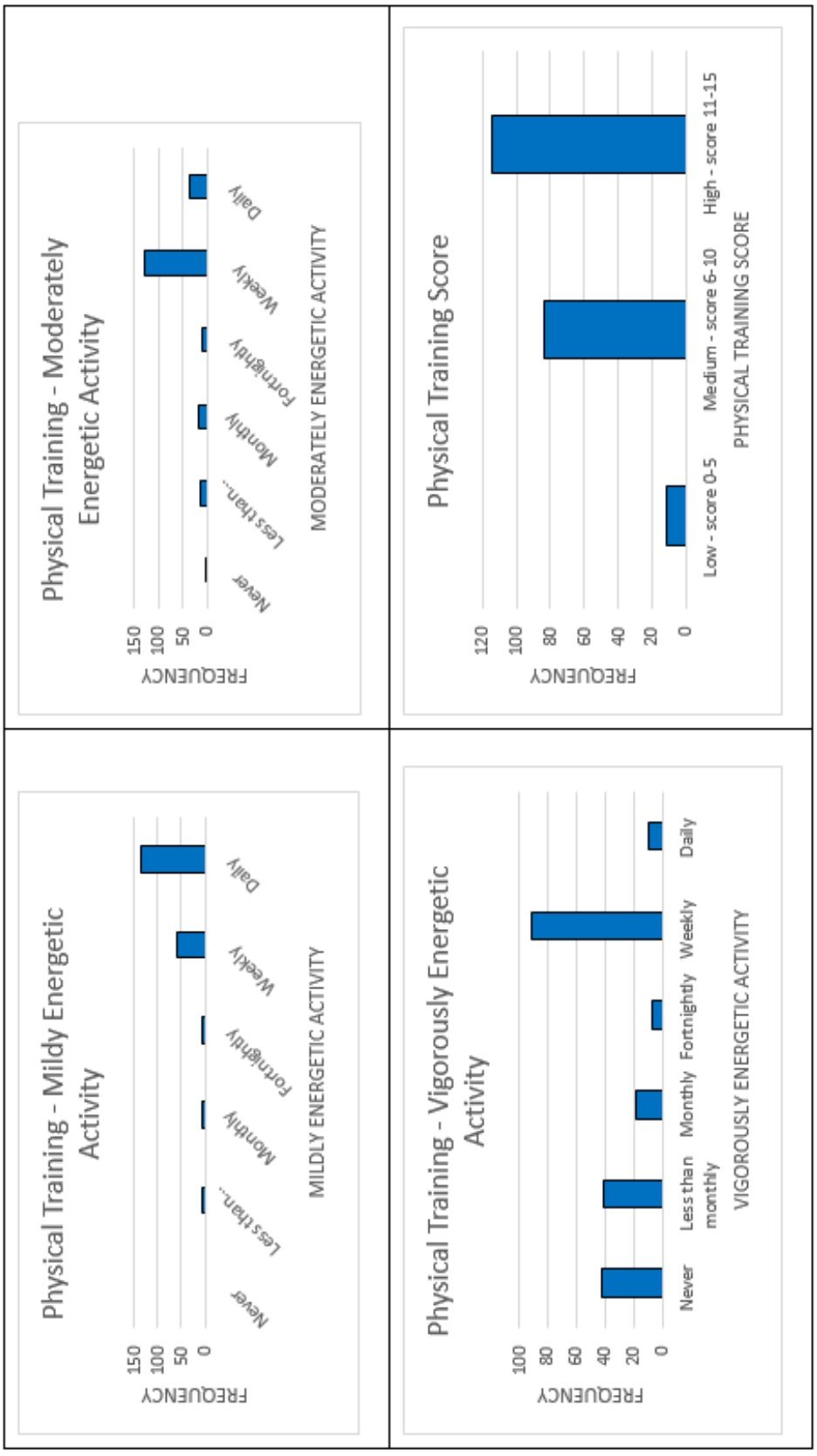

Figure 8: Prevent Dementia cohort physical training score characteristics

Neurodegenerative lifestyle factors

Alcohol

Figure 9 shows the PREVENT Dementia cohort (119) alcohol data. The PREVENT case report form, medical history assessment identified four participants with a diagnosis of alcoholism. The PREVENT case report form, lifestyle assessment identified 183 current drinkers, 5 ex-drinkers and 22 non-drinkers. For current and ex-drinkers, the mean amount of alcohol units consumed per week is 11.7 (S.D 13.2), which is in accordance with being a moderate alcohol drinker, although the large standard deviation indicates that the amount of alcohol units consumed are spread over a wide range of values. This is demonstrated in Figure 9 that shows that the distribution for the number of alcohol units consumed per week is positively skewed.

Figure 9: Prevent Dementia cohort alcohol characteristics

Smoking

Figure 10 shows the PREVENT Dementia cohort (119) smoking data. Only a small number of participants (5%) were current smokers at the time of the PREVENT Dementia cohort (119) baseline assessment. There were 10 current smokers, 80 ex-smokers and 119 non-smokers. For the current and ex-smokers, the mean number of cigarettes smoked per week is 10.9 (SD 8.0) and the range is 1 to 30.

Substance misuse

Figure 10 shows the PREVENT Dementia cohort (119) substance misuse data. In the PREVENT case report form medical history assessment, 5 participants with a diagnosis of substance abuse were identified. From the the PREVENT case report form lifestyle assessment, 4 current substance misusers, 66 ex-substance misusers and 139 participants who had never misused substances were identified. To be categorised as an ex-substance misuser, participants only had to misuse substances on at least one occasion. In the PREVENT programme (119), the substances most commonly misused were cannabis by 63 participants, stimulants by 21 participants, cocaine by 19 participants and hallucinogens by 12 participants.

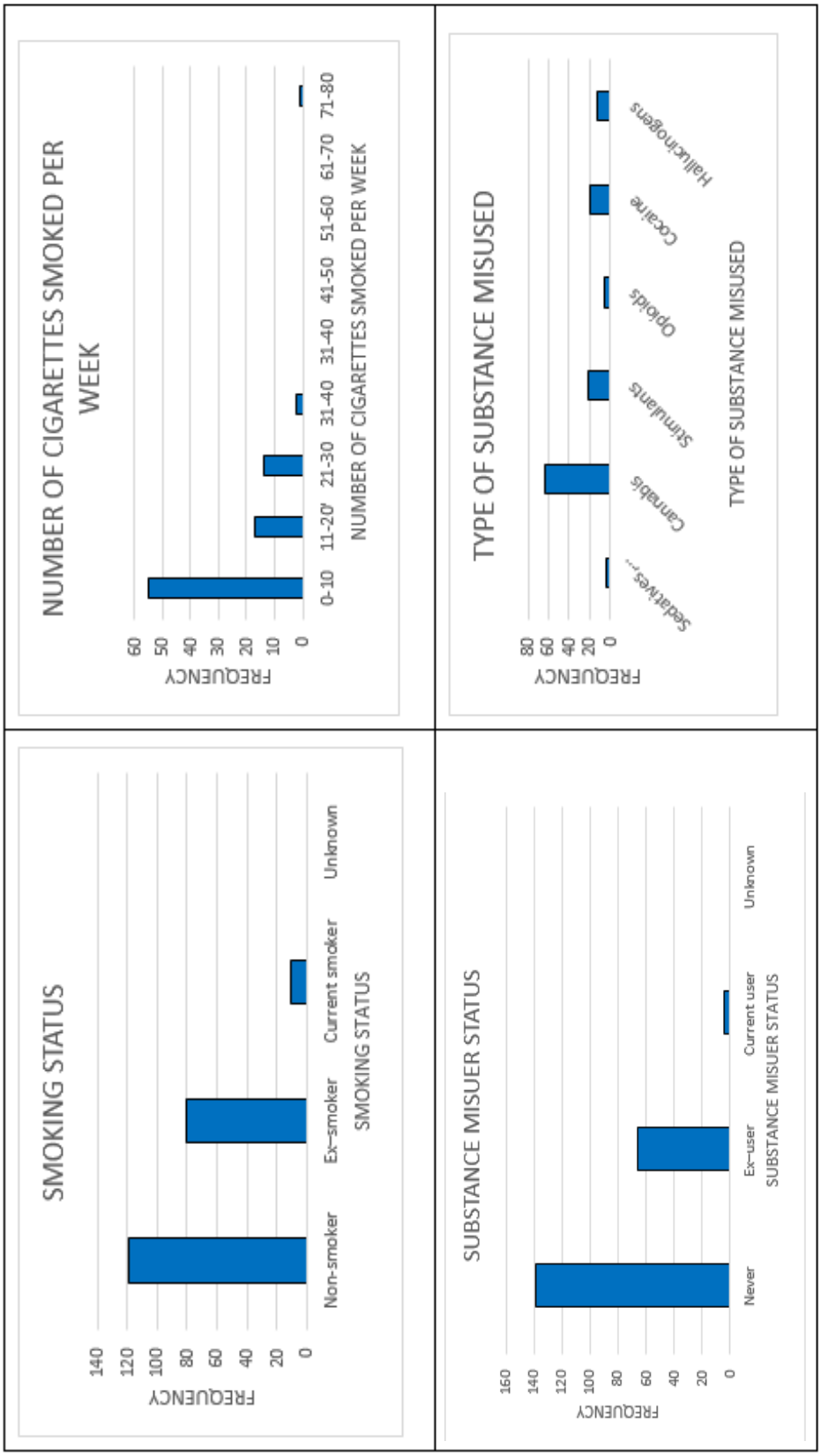

Figure 10: Prevent Dementia cohort smoking and substance misuse characteristics.

3.3.3 Arterial Spin Labelling descriptive statistics

Of the 210 participants in the PREVENT Dementia cohort (119), 193 had MRI scans including ASL for assessment of CBF completed and analysed. There were 17 participants who did not have MRI scans completed. Of note, there were eight participants that did not have MRI scans due to claustrophobia.

Of the 193 who had MRI scans completed; 31 ASL scans were excluded at the image processing stage. There were 18 ASL scans excluded due to having an inadequate field of view where regions of interest were not clearly visualised; six scans were excluded due to the presence of artefact; three scans were excluded as the image files did not open; three were excluded as individual CBF maps did not co-register with the processed individual T1 images; one scan was excluded due to the presence of a meningioma. Therefore, ASL data was available for 162 PREVENT Dementia cohort (119) participants.

The mean value for the mean cortex CBF is 40.1 mL/100g/min and standard deviation 10.2 mL/100g/min. This is in line with the global CBF values identified in the systematic review. In studies by Chapman et al. (85, 86, 113) the mean global CBF values were 47.1 mL/100g/min (113), 47.2 mL/100g/min (85) and 47.0 mL/100g/min(86). The hippocampal CBF mean is 48.3 mL/100g/min and standard deviation is 13.0 mL/100g/min. The ACC CBF mean is 16.9 mL/100g/min and standard deviation is 13.7 mL/100g/min. The CBF data is shown in Figure 11.

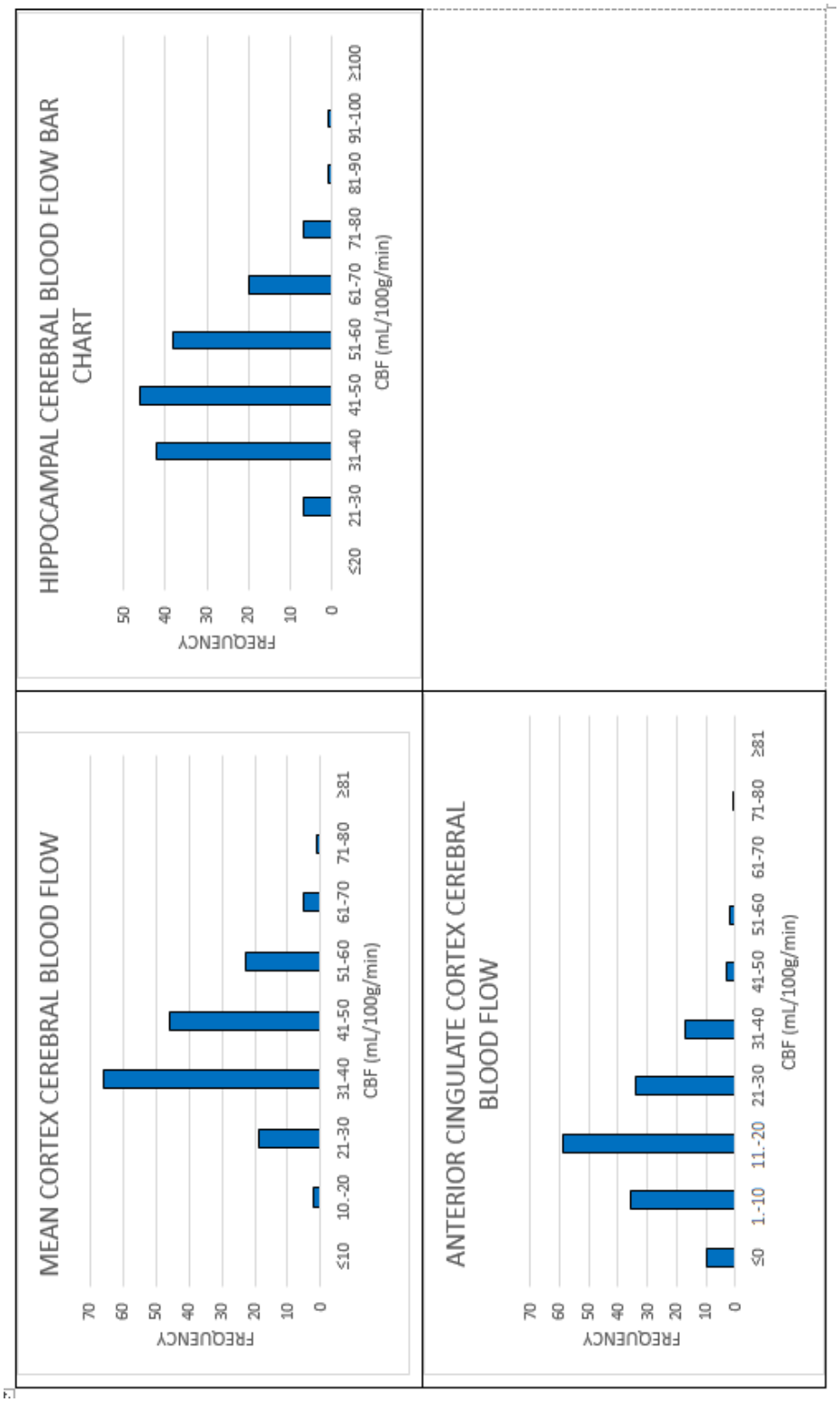

Figure 11: Cerebral blood flow bar charts.

3.3.4 Missing data

Table 20 summarises the missing data from the PREVENT Dementia cohort (119). There was little missing data related to demographics and lifestyle.

Category	Number of participants with missing data
Demographic	
Age	0
Gender	0
APOE ε4 allele	2
Race	0
Number of years of education	0
Domiciliary status	0
Lifestyle	
Physical training score	0
Alcohol intake status	0
Smoking status	1
Substance misuse status	1

Table 20: Missing data from the PREVENT Dementia cohort

3.3.5 Statistical Analysis

The variables used in the statistical models to assess the relationship between lifestyle and neurodegeneration in midlife as expressed on ASL are summarized in Table 21. Taking into account the individuals for whom there was not ASL data and missing data, the sample size was 161.

Table 21: Covariates for the statistical models used to assess the relationship between lifestyle and neurodegeneration in midlife as expressed on ASL.

Statistical model	Covariates
Unadjusted model	
Adjusted linear regression model 1	Age + gender + years of education + *APOE* ε4 allele + number of parents with dementia.
Adjusted linear regression model 2	Age + gender + years of education + *APOE* ε4 allele + number of parents with dementia + physical training score + smoking status + alcohol drinking status + substance misuse status.

3.3.6 Linear regression assumptions

Assumptions were checked for all statistically significant linear regression models and were acceptable. Of note, cases 81, 82 and 175 are outliers, however Cook's distance is less than one and therefore the cases have been included in the analysis. The diagnostic plots (Residuals vs Fitted, Normal Q-Q, Scale-Location, Residuals vs Leverage) are shown in Appendix F.

3.3.7 Linear regression model findings

Physical Training

The results from the linear regression models for physical training are presented in Table 22. Although estimates of the univariate association between physical training and midlife ASL outcomes do not emerge as statistically significant, there is a positive trend in the direction of the association between a low physical training score and all midlife ASL outcomes (Mean cortex CBF, Hippocampal CBF and ACC CBF). This could represent an early neurodegenerative effect or a neuroprotective effect

associated with a low physical training score. The results regarding the association between medium physical training and midlife ASL outcomes do not emerge as statistically significant, nor do they show a clear direction of association.

Table 22: The relationship between physical training score and ASL outcomes in midlife.

Physical training Score	ASL measure	Model	β coefficient (S.E)	p
Low	Mean cortex CBF	Unadjusted Model	0.23 (3.35)	0.94
		Adjusted (Model 1)	0.88 (3.24)	0.79
		Adjusted (Model 2)	0.39 (3.32)	0.91
High	Mean cortex CBF		0 (Reference)	
Low	Hippocampal CBF	Unadjusted Model	2.99 (4.24)	0.48
		Adjusted (Model 1)	3.68 (4.20)	0.38
		Adjusted (Model 2)	2.94 (4.33)	0.50
High	Hippocampal CBF		0 (Reference)	
Low	ACC CBF	Unadjusted Model	3.93 (4.47)	0.38
		Adjusted (Model 1)	4.59 (4.43)	0.30
		Adjusted (Model 2)	4.72 (4.46)	0.29
High	ACC CBF		0 (Reference)	
Medium	Mean cortex CBF	Unadjusted Model	0.08 (1.68)	0.96
		Adjusted (Model 1)	-0.92 (1.67)	0.58
		Adjusted (Model 2)	-1.14 (1.74)	0.51
High	Mean cortex CBF		0 (Reference)	
Medium	Hippocampal CBF	Unadjusted Model	-1.30 (2.13)	0.54
		Adjusted (Model 1)	-2.16 (2.17)	0.32
		Adjusted (Model 2)	-2.17 (2.27)	0.34
High	Hippocampal CBF		0 (Reference)	
Medium	ACC CBF	Unadjusted Model	1.02 (2.25)	0.65
		Adjusted (Model 1)	0.65 (2.30)	0.78
		Adjusted (Model 2)	1.87 (2.33)	0.42
High	ACC CBF		0 (Reference)	

ACC = Anterior cingulate cortex, CBF = Cerebral blood flow

Alcohol

The results from the linear regression models for alcohol are presented in Table 23. The results regarding the association between being a high consumption alcohol drinker and midlife ASL outcomes do not emerge as statistically significant. On adjusted model 2, the results show that compared to being a moderate consumption alcohol drinker, individuals who do not drink alcohol have a statistically significant ($p = 0.04$) reduction in ACC CBF value of -7.62 mL/100g/min (S.E 3.64). The effect size is medium (Pearson's correlation coefficient = 0.37). This could represent a possible neuroprotective effect associated with moderate alcohol intake.

Table 23: The relationship between alcohol drinking status and ASL outcomes in midlife

Alcohol drinking status	ASL measure	Model	β coefficient (S.E)	p
Alcohol drinker – high	Mean cortex CBF	Unadjusted Model	-1.12 (2.02)	0.58
		Adjusted (Model 1)	-1.82 (2.00)	0.36
		Adjusted (Model 2)	-2.25 (2.06)	0.28
Alcohol drinker - moderate	Mean cortex CBF		0 (Reference)	
Alcohol drinker – high	Hippocampal CBF	Unadjusted Model	-2.40 (2.56)	0.35
		Adjusted (Model 1)	-2.94 (2.59)	0.26
		Adjusted (Model 2)	-3.35 (2.69)	0.22
Alcohol drinker - moderate	Hippocampal CBF		0 (Reference)	
Alcohol drinker – high	ACC CBF	Unadjusted Model	-0.36 (2.71)	0.90
		Adjusted (Model 1)	-1.88 (2.74)	0.49
		Adjusted (Model 2)	-2.36 (2.77)	0.40
Alcohol drinker - moderate	ACC CBF		0 (Reference)	
Non-drinker	Mean cortex CBF	Unadjusted Model	-2.90 (2.69)	0.28
		Adjusted (Model 1)	-3.11 (2.63)	0.24
		Adjusted (Model 2)	-3.49 (2.71)	0.20
Alcohol drinker - moderate	Mean cortex CBF		0 (Reference)	
Non-drinker	Hippocampal CBF	Unadjusted Model	-2.08 (3.42)	0.55
		Adjusted (Model 1)	-1.71 (3.43)	0.62
		Adjusted (Model 2)	-2.18 (3.54)	0.54
Alcohol drinker - moderate	Hippocampal CBF		0 (Reference)	
Non-drinker	ACC CBF	Unadjusted Model	-5.71 (3.58)	0.11
		Adjusted (Model 1)	-5.86 (3.59)	0.10
		Adjusted (Model 2)	**-7.62 (3.64)**	**0.04***
Alcohol drinker - moderate	ACC CBF		0 (Reference)	

ACC = Anterior cingulate cortex, CBF = Cerebral blood flow

***=P ≤0.05**

Smoking

The results from the linear regression models for smoking are presented in Table 24. The results do not emerge as statistically significant. The p values range from 0.23 to 0.70. Of note, there is a negative trend in the direction of the association between being a smoker and all midlife ASL outcomes, a result that suggests that smokers have worse ASL outcomes. However a significant result may not have resulted from the analysis undertaken in this thesis due to insufficient power.

Table 24: The relationship between smoking and ASL outcomes in midlife.

Smoking status	ASL measure	Model	β coefficient (S.E)	p
Smoker	Mean cortex CBF	Unadjusted Model	-1.41 (1.63)	0.39
		Adjusted (Model 1)	-0.98 (1.59)	0.54
		Adjusted (Model 2)	-2.25 (1.87)	0.23
Non-smoker	Mean cortex CBF		0 (Reference)	
Smoker	Hippocampal CBF	Unadjusted Model	-1.17 (2.07)	0.57
		Adjusted (Model 1)	-0.79 (2.07)	0.70
		Adjusted (Model 2)	-1.46 (2.44)	0.55
Non-smoker	Hippocampal CBF		0 (Reference)	
Smoker	ACC CBF	Unadjusted Model	-1.59 (2.18)	0.47
		Adjusted (Model 1)	-1.08 (2.19)	0.62
		Adjusted (Model 2)	-1.95 (2.51)	0.44
Non-smoker	ACC CBF		0 (Reference)	

ACC = Anterior cingulate cortex, CBF = Cerebral blood flow

Substance misuse

The results from the linear regression models for substance misuse are presented in Table 25. Estimates of the univariate and multivariate association between substance misuse and both mean cortex cerebral blood flow and hippocampal blood flow do not emerge as statistically significant nor do they show a trend in the direction of association.

On all three linear regression models, the results show that compared to being a non-substance misuser, being a current or ex-substance misuser is associated with a reduced ACC CBF. This is likely to represent a neurodegenerative effect associated with substance misuse. On the unadjusted model, there is a statistically significant ($p = 0.02$) reduction in ACC CBF of -5.13 mL/100g/min (S.E 2.25). The effect size is small (Pearson's correlation coefficient = 0.18). On adjusted model 1, individuals who are current or ex-substance misusers have a statistically significant ($p = 0.02$) reduction in ACC CBF of -5.58 mL/100g/min (S.E 2.33). Again, the effect size is small (Pearson's correlation coefficient = 0.29). On adjusted model 2, there is a statistically significant ($p = 0.02$) reduction in ACC CBF of -5.58 mL/100g/min (S.E 2.33) and the effect size is medium (Pearson's correlation coefficient = 0.37).

Table 25: The relationship between substance misuse and ASL outcomes in midlife.

Substance misuse status	ASL measure	Model	β coefficient (S.E)	p
Current or ex-substance misuser	Mean cortex CBF	Unadjusted Model	1.41 (1.70)	0.41
		Adjusted (Model 1)	1.88 (1.72)	0.28
		Adjusted (Model 2)	3.15 (2.01)	0.12
Never misused substances	Mean cortex CBF		0 (Reference)	
Current or ex-substance misuser	Hippocampal CBF	Unadjusted Model	1.87 (2.16)	0.39
		Adjusted (Model 1)	1.60 (2.24)	0.48
		Adjusted (Model 2)	2.93 (2.63)	0.27
Never misused substances	Hippocampal CBF		0 (Reference)	
Current or ex-substance misuser	**ACC CBF**	**Unadjusted Model**	**-5.13 (2.25)**	**0.02***
		Adjusted (Model 1)	**-5.58 (2.33)**	**0.02***
		Adjusted (Model 2)	**-7.29 (2.70)**	**<0.01***
Current or ex-substance misuser	ACC CBF		0 (Reference)	

ACC = Anterior cingulate cortex, CBF = Cerebral blood flow

***=P ≤0.05**

3.4 Discussion

The discussion of the analysis of the PREVENT Dementia cohort (119) data is presented below and the potential mechanisms and clinical and public health implications are presented in chapter 5.

3.4.1 Main findings

The main finding of this study is an association between being a current or ex-substance misuser and a reduction in ACC CBF. The ACC as expressed on fMRI is illustrated in Figure 12. Additionally, the results from adjusted model 2 show that compared to being a moderate alcohol drinker, being a non-drinker is associated with a reduction in ACC CBF. Linear regression models did not show an association between physical training and smoking with mean cortex CBF, hippocampal CBF and ACC CBF.

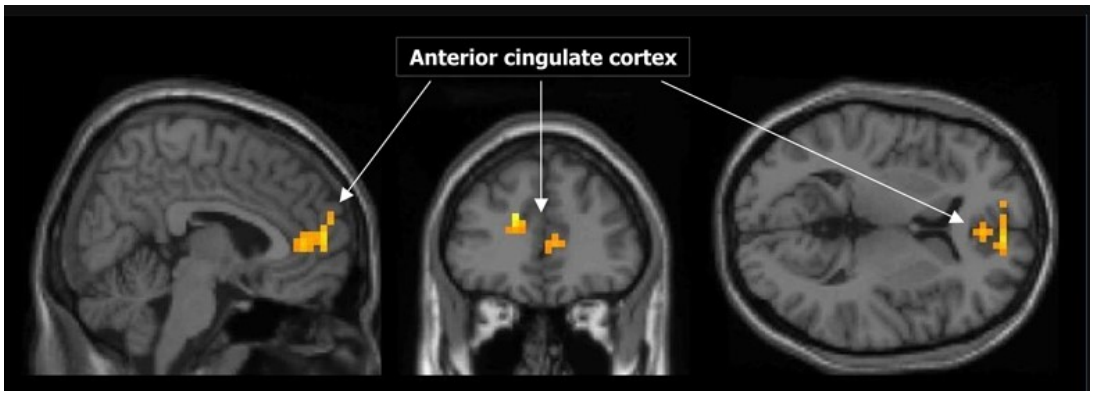

Figure 12: Anterior Cingulate Cortex on functional Magnetic Resonance Imaging (165).

3.4.2 Comparison with other literature

The studies showing a relationship between lifestyle and neurodegeneration in midlife as expressed on fMRI are summarised in Chapter 2. Please see section 2.3.3 (Studies included in qualitative

synthesis) for full details. Multiple studies identified by the systematic review have shown a relationship between lifestyle factors and neurodegeneration in the ACC in midlife as expressed on fMRI. Of note, in a cross-sectional, resting state fMRI study of 24 chronic heroin users and 24 normal controls, Jiang et al. demonstrated decreased amplitude low-frequency fluctuate (ALFF), a measure of abnormal activity, in multiple brain regions. More specifically, the study found that compared with controls, individuals who were addicted to heroin had decreased ALFF in the ACC, which is thought to be abnormal (94). Furthermore, in a cross-sectional multi-modal MRI study of 20 cocaine-addicted individuals and 19 age-matched controls, Wang et al. demonstrated that compared to controls, individuals addicted to cocaine showed a hypo-status in multiple brain regions. Amongst these results, the ACC showed a correlation between high drug craving scores and low tissue volume and low CBF. (107). Using data from the PREVENT Dementia cohort (119), an assessment of the correlation between fMRI and sMRI findings would be helpful to determine if a reduced ACC CBF corresponds to reduced ACC tissue volume in substance misusers, however this data was not available for my analysis.

Although the systematic review identified six studies (88, 90, 96, 105, 106, 108) that show an association between high alcohol consumption and neurodegeneration, the association found in this data analysis between being a non-drinker and a reduction in the ACC CBF was not identified by the systematic review.

The systematic review identified five studies that showed a potential neuroprotective effect associated with physical training (84, 86, 99, 101, 109), one study that showed no effect (91) and one study that described a possible neurodegenerative effect (89). In the study that showed no effect, Hotting et al. (91) assessed the effects of cognitive training and physical training on spatial learning and associated brain activation. The physical training interventions involved indoor-cycling on stationary bicycles and stretching and coordination training. Spatial learning was assessed with a virtual maze task, and at the same time neural correlates were measured with fMRI. The physical intervention group did not improve performance in the maze task. In line with this study, the analysis of the PREVENT Dementia cohort (119) did not show an association between physical training and CBF as expressed on fMRI. However, the measure of physical training, used in my analysis, a physical training score based on frequency and intensity of physical training is different to the physical training intervention used in the study by Hotting et al. (91). Furthermore fMRI outcome measures used in my analysis - mean cortex CBF, hippocampal CBF and ACC CBF, are different to the fMRI outcome measure used in the study by Hotting et al. (91). The systematic review identified three cross-sectional studies (87, 88, 103) that demonstrated a neurodegenerative effect associated with smoking on brain health as expressed on fMRI. This finding was not replicated in my data analysis which did not show an association between smoking and mean cortex CBF, hippocampal CBF and ACC CBF.

3.4.3 Linear regression model covariates

In accordance with guidance by Smith, the covariates for the linear regression models have been stated a priori and stepwise regression has not been undertaken (166). The reason for this is that in stepwise regression, some real explanatory variables that have causal effects on the dependent variable may happen to not be statistically significant, while nuisance variables may be coincidentally significant. As a result, the model may fit the data well in-sample, but do poorly out of sample (166). The potential for bias when covariates are chosen by data dependent methods has been acknowledged by the International Conference on Harmonization (ICH) guidelines on statistical principles for clinical trials (167).

The decision as to which covariates to include in the analysis were based on data from studies identified in the systematic review of the relationship between lifestyle and neurodegeneration in midlife as expressed on fMRI and established non-modifiable risk factors for Alzheimer's dementia. However it is worth bearing in mind that stating the linear regression coefficients a priori, introduces the risk of overlooking a hidden confounder that has not been anticipated. (168)

3.4.4 Strengths and limitations

Important limitations of this cross-sectional data analysis of the PREVENT Dementia cohort data (119) are that causation cannot be inferred and that low power may have contributed to a reduced chance of detecting a true effect in the statistical analysis (169). Based on guidance by Cohen on statistical power analysis (170), the standard α-level of 0.05 and the

recommended power of 0.8 have been used, therefore 783 participants would be needed to detect a small effect size, 85 participants to detect a medium effect size and 28 participants to detect a large effect size. In this data analysis, consideration was given to using the Bonferroni adjustment to reduce the possibility of a Type I error. However, the decision was made not to use the Bonferroni adjustment, as it implies that a given comparison will be interpreted differently according to how many other tests were performed and although it would decrease the number of type I errors, it would increase the number of type II errors (171). Therefore in accordance with guidance by Perneger (171), the approach that has been adopted is to describe what analysis has been undertaken and why. Then the possible interpretations of each result have been explored. The intention of this approach is to enable the reader to reach a reasonable conclusion without the help of the Bonferroni adjustment.

A further limitation of this analysis is the use of self-reported measures of physical training, alcohol, smoking and substance misuse in the PREVENT Dementia cohort (119). Self-reported measures are a source of response bias. Additionally, the PREVENT Dementia cohort (119) physical training score which takes into account the frequency and intensity of physical training, has not been validated in past literature. It is noteworthy that individuals who volunteered to be part of the PREVENT Dementia cohort (119) baseline population in London were mainly Caucasian (90%) and highly educated; the mean number of years of education was 16. This limits the generalisability of the findings of this cross- sectional analysis. An

important strength of this analysis is the large number of participants for whom ASL data has been collected and assessed. This compares favourably to 27 of the 29 studies (81-92, 94-107, 109) identified in the systematic review. Furthermore there is little missing data in the PREVENT Dementia cohort (119) data analysis.

3.4.5 Conclusions

Previous studies have shown a relationship between multiple lifestyle factors – substance misuse, smoking, alcohol, physical and cognitive training and neurodegeneration in the ACC in midlife as expressed on fMRI. The analysis of the data from the PREVENT Dementia cohort (119) has demonstrated associations between substance misuse and alcohol, and ASL CBF in the ACC. No associations between physical training and smoking, and mean cortex CBF, hippocampal CBF and ACC CBF were detected.

3.4.6 Implications of research

This analysis of the PREVENT Dementia cohort (119), demonstrated associations between being a current or ex-substance misuser and ACC CBF and not drinking alcohol and ACC CBF. As the analysis is cross sectional, causation cannot be inferred and it is worth emphasizing that the results are of limited generalisability. This is partly due to the inclusion criteria for the cohort and the participant characteristics. The inclusion criteria means the cohort only includes participants aged 40 to 59 years old. The participant characteristics show that the participants in the PREVENT Dementia cohort (119) are a select group of volunteers who are mainly Caucasian, highly education and physically fit.

4 Analysis of the relationship between lifestyle and structural magnetic resonance imaging outcomes in midlife using data from the PREVENT Dementia cohort

Chapter abstract

INTRODUCTION: The linear regression models used to analytically assess the relationship between lifestyle factors and neurodegeneration in midlife, as expressed using ASL, were adapted to undertake analysis of the relationship between lifestyle and neurodegeneration in midlife as expressed on structural MRI (sMRI) using data from the PREVENT Dementia cohort.

METHODS: Three linear regression models were constructed to evaluate the relationship between lifestyle and neurodegeneration as expressed on sMRI. The first model adjusted for intracranial volume. The second model also took into account non modifiable risk factors for dementia. The third model adjusted for intracranial volume, non modifiable risk factors for dementia and lifestyle factors.

RESULTS: There were 193 individuals in the PREVENT Dementia cohort with sMRI data. The linear regression models demonstrated three key associations - a medium physical training score was associated with an increased hippocampal grey matter volume, a low physical training score was associated with an increased PVH Fazekas score and being a smoker was associated with reduced grey matter volume.

DISCUSSION: This exploratory analysis represents a preliminary step in the investigation of the relationship between lifestyle factors and neurodegeneration in midlife as expressed on sMRI. There is scope to build on this exploratory analysis by completing a systematic review of the relationship between lifestyle and neurodegeneration in midlife as expressed on sMRI and refinement of the linear regression models.

4.1 Introduction

The systematic review (chapter 2) identified five key lifestyle factors – physical training, cognitive training, smoking, alcohol and substance misuse associated with neurodegeneration. Based on the findings of the systematic review, linear regression models were constructed to analytically assess the relationship between lifestyle and neurodegeneration in midlife as expressed on ASL using the PREVENT Dementia cohort data (119) (chapter 3). The ASL linear regression models were then optimised to undertake analysis of the relationship between lifestyle and neurodegeneration in midlife as expressed on sMRI using the PREVENT Dementia cohort data (119). The following hypothesis were tested:

1. Physical training is associated with increased GMV, WMV, hippocampal grey matter volume and parahippocampal grey matter volume.
2. Physical training is associated with a decreased periventricular hyperintensities (PVH) Fazekas score and deep white matter hyperintensities (DWMH) Fazekas score (158).
3. Substance misuse, alcohol and smoking are associated with decreased GMV, WMV, hippocampal grey matter volume and parahippocampal grey matter volume.
4. Substance misuse, alcohol and smoking are associated with an increased PVH and DWMH Fazekas score (158).

4.2 Method

Baseline data from the PREVENT Dementia cohort (119) were used to assess the relationship between lifestyle and neurodegeneration in midlife as expressed on sMRI. A full description of the PREVENT Dementia cohort (119) is available in Section 3.2.1 (The Prevent Dementia cohort). The covariates used in the linear regression models have been chosen in advance based on the systematic review of the relationship between lifestyle and neurodegeneration in midlife as expressed on fMRI and established non-modifiable risk factors for Alzheimer's dementia. Additionally, intracranial volume (ICV) was used as a covariate. This has been included as normalization of regional volumes by ICV can aid the assessment of neurodegenerative changes on sMRI (172).

The covariates used in the statistical models to assess the relationship between lifestyle and neurodegeneration in midlife as expressed on sMRI are shown in Table 26. Three linear regression models were constructed and the covariates used were ICV, age, gender, years of education, *APOE* ε4 allele, number of parents with dementia, physical training score, smoking status, alcohol drinking status and substance misuse status. The sMRI outcome measures assessed were the available sMRI measures from the Prevent Dementia cohort (119) - GMV, WMV, hippocampal grey matter volume, parahippocampal grey matter volume, PVH Fazekas score and DWMH Fazekas score (158).

Table 26: Covariates for the statistical models used to assess the relationship between lifestyle and neurodegeneration in midlife as expressed on structural MRI.

Statistical model	Covariates
Unadjusted linear regression model	ICV.
Adjusted linear regression model 1	ICV + age + gender + years of education + *APOE* ε4 allele + number of parents with dementia.
Adjusted linear regression model 2	ICV + age + gender + years of education + *APOE* ε4 allele + number of parents with dementia + physical training score + smoking status + alcohol drinking status + substance misuse status.

ICV = Intracranial volume, APOE = Apolipoprotein

4.3 Results

The structural MRI summary statistics for the 193 participants in the PREVENT Dementia cohort (119) with sMRI data are presented in Table 27. The table shows the mean, median and range for GMV, WMV, hippocampal grey matter volume, parahippocampal grey matter volume, PVH Fazekas score and DWMH Fazekas score (158).

Table 27: Structural MRI summary statistics.

Summary measures	
GMV mean ± SD	642.9 ± 56.07 cm^3
GMV median / Interquartile range	638 / 73.8 cm^3
GMV range	478.4 to 842.9 cm^3
WMV mean ± SD	516.3 ± 61.78 cm^3
WMV median / Interquartile range	515 / 74.2 cm^3
WMV range	346.6 to 683.9 cm^3
ICV mean ± SD	1389 ± 131.11 cm^3
ICV median / Interquartile range	1382 / 179.1 cm^3
ICV range	1088 to 1804 cm^3
Hippocampal grey matter volume mean ± SD	3.73 ± 0.34
Hippocampal grey matter volume median / Interquartile range	3.69 / 0.45
Hippocampal grey matter volume range	2.77 to 4.71
Parahippocampal grey matter volume mean ± SD	3.73 ± 0.35
Parahippocampal grey matter volume median / Interquartile range	3.68 / 0.50
Parahippocampal grey matter volume range	2.98 to 4.64
PVH mean ± SD	1.13 ± 0.42
DWMH mean ± SD	0.68 ± 0.57

DWMH = Deep white matter hyperintensities, ICV = Intracranial volume, GMV = Grey matter volume, PVH = Periventricular hyperintensities, SD = Standard deviation, WMV = White matter volume

Participant characteristics

The PREVENT Dementia cohort (119) included 210 participants who had completed the baseline visit at the pilot site. Of the 210 participants, 193 had MRI data available. There were 17 participants who did not complete the MRI scan. This was due to claustrophobia (n=8), withdrawal of consent (n=1), did not attend (n=1), metal implant (n=1), scanner not available (n=1) and other reasons not specified (n=5).

The descriptive characteristics for both the full (N=210) and analysed data (N=193) sets are shown in Table 28. There were no significant differences between the demographics of the full data set and the analysed data set. In the analysed data set, the average age of participants was 52 years old. There were 58 (30%) male and 135 (70%) female participants. There were 70 (36.3%) participants who had at least one *APOE* ε4 allele and 121 (62.7%) participants had no *APOE* ε4 allele. The mean number of years of education was 15.9.

Table 28: Summary of Prevent Dementia cohort demographic characteristics.

Demographics	N=210 (full data set)	N=193 (analysed data set)
Age - mean ± SD	52.0 ± 5.5	52.0 ± 5.4
Gender: M / F - N (% of total sample)	62 (29.5) / 148 (70.5)	58 (30) / 135 (70)
APOE ε4 allele: No *APOE* ε4 / *APOE* ε4 ≥ 1 – N (% of total sample)	133 (63.9) / 75 (36.1)	121 (62.7) / 70 (36.3)
Race: Caucasian / other - N (% of total sample)	188 (89.5) / 22 (10.5)	175 (91) / 18 (9)
Number of years of education: mean + SD	15.9 ± 3.4	15.9 ± 3.4
Domiciliary status: lives with others / lives alone – N (% of total sample)	180 (85.7) / 30 (14.3)	166 (86) / 27 (14)

Low Physical Training Score

The results from the linear regression models for a low physical training score are presented in Table 29. Although estimates of the association between physical training and volumetric sMRI outcomes (GMV, WMV, Hippocampal volume, Parahippocampal volume) do not emerge as statistically significant, there is a negative trend in the direction of the association. Contrastingly there is a positive trend in the direction of association between physical training and Fazekas scores (PVH and DWMH) (158). Furthermore, on all models, the results show that compared to having a high physical training score, a low physical training score is associated with a statistically significant higher PVH Fazekas score (158). A higher PVH Fazekas score (158) represents increased white matter lesions, which suggests a reduction in brain health.

Table 29: The relationship between physical training score (low vs high) and structural MRI outcomes in midlife.

Physical training Score	sMRI measure	Model	β coefficient (S.E)	p
Low	GMV	Unadjusted model	-8.71 (6.97)	0.21
		Adjusted (Model 1)	-7.80 (6.84)	0.26
		Adjusted (Model 2)	-10.81 (6.87)	0.12
High	GMV		0 (Reference)	
Low	WMV	Unadjusted model	-7.92 (9.13)	0.39
		Adjusted (Model 1)	0.77 (3.64)	0.83
		Adjusted (Model 2)	-4.13 (3.40)	0.23
High	WMV		0 (Reference)	
Low	Hippocampal volume	Unadjusted model	-0.04 (0.06)	0.5
		Adjusted (Model 1)	-0.05 (0.06)	0.46
		Adjusted (Model 2)	-0.07 (0.062)	0.23
High	Hippocampal volume		0 (Reference)	
Low	Parahippocampal volume	Unadjusted model	-0.05 (0.06)	0.47
		Adjusted (Model 1)	-0.04 (0.06)	0.49
		Adjusted (Model 2)	-0.03 (0.07)	0.66
High	Parahippocampal volume		0 (Reference)	
Low	**PVH Fazekas score**	**Unadjusted model**	**0.45 (0.13)**	**<0.01***
		Adjusted (Model 1)	**0.44 (0.12)**	**<0.01***
		Adjusted (Model 2)	**0.45 (0.13)**	**<0.01***
High	PVH Fazekas score		0 (Reference)	
Low	DWMH Fazekas score	Unadjusted model	0.04 (0.18)	0.81
		Adjusted (Model 1)	0.06 (0.18)	0.72
		Adjusted (Model 2)	0.06 (0.18)	0.75
High	DWMH Fazekas score		0 (Reference)	

DWMH = Deep white matter hyperintensities, GMV = Grey matter volume, PVH = Periventricular hyperintensities, S.E = Standard error, WMV = White matter volume

***=P ≤0.05**

Medium Physical Training Score

The results from the linear regression models for a medium physical training score are presented in Table 30. Estimates of the association between physical training and sMRI outcomes do not show a general clear trend in the direction of association. However, on all the models, the results show that compared to having a high physical training score, a medium physical training score is associated with a reduction in hippocampal volume, which likely represents neurodegeneration.

Table 30: The relationship between medium physical training score (medium vs high) and structural MRI outcomes in midlife.

Physical training score	sMRI measure	Model	β coefficient (S.E)	p
Medium	GMV	Unadjusted model	-4.26 (3.32)	0.20
		Adjusted (Model 1)	-3.41 (3.37)	0.31
		Adjusted (Model 2)	-4.13 (3.40)	0.23
High	GMV		0 (Reference)	
Medium	WMV	Unadjusted model	0.17 (3.71)	0.96
		Adjusted (Model 1)	0.77 (3.64)	0.83
		Adjusted (Model 2)	0.58 (3.78)	0.88
High	WMV		0 (Reference)	
Medium	**Hippocampal volume**	**Unadjusted model**	**-0.07 (0.03)**	**0.02***
		Adjusted (Model 1)	**-0.07 (0.03)**	**0.03***
		Adjusted (Model 2)	**-0.07 (0.03)**	**0.02 ***
High	Hippocampal volume		0 (Reference)	
Medium	Parahippocampal volume	Unadjusted model	0.01 (0.03)	0.74
		Adjusted (Model 1)	0.02 (0.03)	0.53
		Adjusted (Model 2)	0.02 (0.03)	0.56
High	Parahippocampal volume		0 (Reference)	
Medium	PVH Fazekas score	Unadjusted model	-0.1 (0.06)	0.83
		Adjusted (Model 1)	0.02 (0.07)	0.75
		Adjusted (Model 2)	0.02 (0.07)	0.80
High	PVH Fazekas score		0 (Reference)	
Medium	DWMH Fazekas score	Unadjusted model	0.03 (0.08)	0.68
		Adjusted (Model 1)	-0.02 (0.09)	0.85
		Adjusted (Model 2)	-0.01 (0.09)	0.95
High	DWMH Fazekas score		0 (Reference)	

DWMH = Deep white matter hyperintensities, GMV = Grey matter volume, PVH = Periventricular hyperintensities, S.E = Standard error, WMV = White matter volume

***=P ≤0.05**

Smoking

The results from the linear regression models for smoking are presented in Table 31. The association between smoking and sMRI outcomes do not show a general clear trend in the direction of association. However, on all the models, the results show that compared to being a non-smoker, being a smoker is associated with a reduction in GMV. Furthermore, on the unadjusted model, individuals who are smokers, have a statistically significant (p=0.03) increase in WMV of 7.82 (3.61). Both these changes likely represent neurodegeneration.

Table 31: The relationship between smoking status (smoker vs non-smoker) and structural MRI outcomes in midlife.

Smoking status	sMRI measure	Model	β coefficient (S.E)	p
Smoker	**GMV**	**Unadjusted model**	**-10.21 (3.19)**	**<0.01***
		Adjusted (Model 1)	**-10.29 (3.19)**	**<0.01***
		Adjusted (Model 2)	**-9.77 (3.75)**	**0.01***
Non-smoker	GMV		0 (Reference)	
Smoker	**WMV**	**Unadjusted model**	**7.82 (3.61)**	**0.03***
		Adjusted (Model 1)	5.92 (3.52)	0.09
		Adjusted (Model 2)	5.46 (4.17)	0.19
Non-smoker	WMV		0 (Reference)	
Smoker	Hippocampal volume	Unadjusted model	0.01 (0.03)	0.61
		Adjusted (Model 1)	0.02 (0.03)	0.41
		Adjusted (Model 2)	0.04 (0.03)	0.20
Non-smoker	Hippocampal volume		0 (Reference)	
Smoker	Parahippocampal volume	Unadjusted model	0.03 (0.03)	0.25
		Adjusted (Model 1)	0.04 (0.03)	0.25
		Adjusted (Model 2)	0.04 (0.04)	0.33
Non-smoker	Parahippocampal volume		0 (Reference)	
Smoker	PVH Fazekas score	Unadjusted model	0.07 (0.06)	0.29
		Adjusted (Model 1)	0.07 (0.06)	0.24
		Adjusted (Model 2)	0.07 (0.07)	0.36
Non-smoker	PVH Fazekas score		0 (Reference)	
Smoker	DWMH Fazekas score	Unadjusted model	0.01 (0.08)	0.86
		Adjusted (Model 1)	0.03 (0.08)	0.74
		Adjusted (Model 2)	0.02 (0.10)	0.81
Non-smoker	DWMH Fazekas score		0 (Reference)	

DWMH = Deep white matter hyperintensities, GMV = Grey matter volume, PVH = Periventricular hyperintensities, S.E = Standard error, WMV = White matter volume

***=P ≤0.05**

High Consumption Alcohol Drinker

The results from the linear regression models for being a high consumption alcohol drinker are presented in Table 32. Estimates of the association between high consumption and volumetric sMRI outcomes (GMV, WMV, Hippocampal volume, Parahippocampal volume) do not show a clear trend in the direction of association. However, there is a positive trend in the direction of association between high consumption and Fazekas scores (PVH and DWMH) (158). Additionally on the unadjusted model, the association between high alcohol consumption and an increased DWMH Fazekas score (158) is approaching significance (p=0.18).

Table 32: The relationship between alcohol drinking status (alcohol drinker – high vs alcohol drinker - moderate) and structural MRI outcomes in midlife.

Alcohol drinking status	sMRI measure	Model	β coefficient (S.E)	p
Alcohol drinker – high	GMV	Unadjusted model	-2.37 (3.85)	0.54
		Adjusted (Model 1)	0.98 (3.51)	0.78
		Adjusted (Model 2)	0.51 (3.89)	0.90
Alcohol drinker – moderate	GMV		0 (Reference)	
Alcohol drinker – high	WMV	Unadjusted model	-1.51 (4.29)	0.72
		Adjusted (Model 1)	0.38 (4.16)	0.93
		Adjusted (Model 2)	-0.50 (4.33)	0.91
Alcohol drinker – moderate	WMV		0 (Reference)	
Alcohol drinker – high	Hippocampal volume	Unadjusted model	0.02 (0.03)	0.47
		Adjusted (Model 1)	0.02 (0.03)	0.51
		Adjusted (Model 2)	0.02 (0.04)	0.61
Alcohol drinker – moderate	Hippocampal volume		0 (Reference)	
Alcohol drinker – high	Parahippocampal volume	Unadjusted model	-0.01 (0.04)	0.89
		Adjusted (Model 1)	-0.01 (0.04)	0.84
		Adjusted (Model 2)	-0.01 (0.04)	0.86
Alcohol drinker – moderate	Parahippocampal volume		0 (Reference)	
Alcohol drinker – high	PVH Fazekas score	Unadjusted model	0.06 (0.07)	0.41
		Adjusted (Model 1)	0.05 (0.07)	0.48
		Adjusted (Model 2)	0.01 (0.08)	0.88
Alcohol drinker – moderate	PVH Fazekas score		0 (Reference)	

DWMH = Deep white matter hyperintensities, GMV = Grey matter volume, PVH = Periventricular hyperintensities, S.E = Standard error, WMV = White matter volume

Alcohol drinking status	sMRI measure	Model	β coefficient (S.E)	p
Alcohol drinker – high	DWMH Fazekas score	Unadjusted model	0.13 (0.10)	0.18
		Adjusted (Model 1)	0.10 (0.1)	0.33
		Adjusted (Model 2)	0.09 (0.10)	0.40
Alcohol drinker – moderate	DWMH Fazekas score		0 (Reference)	

DWMH = Deep white matter hyperintensities, GMV = Grey matter volume, PVH = Periventricular hyperintensities, S.E = Standard error, WMV = White matter volume

Non-drinker

The results from the linear regression models for being a non-drinker are presented in Table 33. The association between non-drinker and sMRI outcomes do not show an obvious trend in the direction of association.

Table 33: The relationship between alcohol drinking status (non-drinker vs alcohol drinker – moderate) and structural MRI outcomes in midlife.

Alcohol drinking status	sMRI measure	Model	β coefficient (S.E)	p
Non-drinker	GMV	Unadjusted model	-3.12 (5.67)	0.58
		Adjusted (Model 1)	0.07 (5.6)	0.99
		Adjusted (Model 2)	-3.10 (5.66)	0.58
Alcohol drinker – moderate	GMV		0 (Reference)	
Non-drinker	WMV	Unadjusted model	1.16 (6.31)	0.85
		Adjusted (Model 1)	2.93 (6.08)	0.63
		Adjusted (Model 2)	4.56 (6.29)	0.47
Alcohol drinker – moderate	WMV		0 (Reference)	
Non-drinker	Hippocampal volume	Unadjusted model	0.01 (0.05)	0.86
		Adjusted (Model 1)	0.01 (0.05)	0.86
		Adjusted (Model 2)	0.01 (0.05)	0.82
Alcohol drinker – moderate	Hippocampal volume		0 (Reference)	
Non-drinker	Parahippocampal volume	Unadjusted model	0.03 (0.05)	0.57
		Adjusted (Model 1)	0.03 (0.05)	0.58
		Adjusted (Model 2)	0.04 (0.05)	0.46
Alcohol drinker – moderate	Parahippocampal volume		0 (Reference)	
Non-drinker	PVH Fazekas score	Unadjusted model	-0.14 (0.11)	0.20
		Adjusted (Model 1)	-0.16 (0.11)	0.14
		Adjusted (Model 2)	-0.14 (0.11)	0.22
Alcohol drinker – moderate	PVH Fazekas score		0 (Reference)	

DWMH = Deep white matter hyperintensities, GMV = Grey matter volume, PVH = Periventricular hyperintensities, S.E = Standard error, WMV = White matter volume

Alcohol drinking status	sMRI measure	Model	β coefficient (S.E)	p
Non-drinker	DWMH Fazekas score	Unadjusted model	-0.03 (0.14)	0.82
		Adjusted (Model 1)	-0.08 (0.14)	0.57
		Adjusted (Model 2)	-0.06 (0.15)	0.71
Alcohol drinker – moderate	DWMH Fazekas score		0 (Reference)	

DWMH = Deep white matter hyperintensities, GMV = Grey matter volume, PVH = Periventricular hyperintensities, S.E = Standard error, WMV = White matter volume

Substance misuse

The linear regression models for the relationship between being a current or ex-substance misuser compared to individuals who never misused substances are presented in Table 34. Of note, on adjusted model 1, individuals who are current or ex-substance misusers, have a statistically significant (p=0.05) reduction in GMV of -6.99 (S.E 3.50). This is likely to represent a pathological change associated with substance misuse.

Table 34: The relationship between substance misuse status (Current or ex-substance misuser vs never misused substances) and structural MRI outcomes in midlife.

Substance misuse status	sMRI measure	Model	β coefficient (S.E)	p
Current or ex-substance misuser	**GMV**	Unadjusted model	-3.77 (3.46)	0.28
		Adjusted (Model 1)	**-6.99 (3.50)**	**0.05***
		Adjusted (Model 2)	-1.80 (4.00)	0.65
Never misused substances	GMV		0 (Reference)	
Current or ex-substance misuser	WMV	Unadjusted model	3.29 (3.87)	0.40
		Adjusted (Model 1)	4.57 (3.81)	0.23
		Adjusted (Model 2)	2.04 (4.45)	0.65
Never misused substances	WMV		0 (Reference)	
Current or ex-substance misuser	Hippocampal volume	Unadjusted model	-0.03 (0.03)	0.33
		Adjusted (Model 1)	-0.02 (0.03)	0.50
		Adjusted (Model 2)	-0.04 (0.04)	0.26
Never misused substances	Hippocampal volume		0 (Reference)	
Current or ex-substance misuser	Parahippocampal volume	Unadjusted model	0.03 (0.03)	0.35
		Adjusted (Model 1)	0.02 (0.03)	0.51
		Adjusted (Model 2)	<0.01 (0.04)	0.90
Never misused substances	Parahippocampal volume		0 (Reference)	
Current or ex-substance misuser	PVH Fazekas score	Unadjusted model	0.03 (0.07)	0.64
		Adjusted (Model 1)	0.05 (0.07)	0.43
		Adjusted (Model 2)	0.01 (0.08)	0.92
Never misused substances	PVH Fazekas score		0 (Reference)	

DWMH = Deep white matter hyperintensities, GMV = Grey matter volume, PVH = Periventricular hyperintensities, S.E = Standard error, WMV = White matter volume

***=P ≤0.05**

Substance misuse status	sMRI measure	Model	β coefficient (S.E)	p
Current or ex-substance misuser	DWMH Fazekas score	Unadjusted model	-0.03 (0.09)	0.77
		Adjusted (Model 1)	-0.01 (0.09)	0.99
		Adjusted (Model 2)	-0.03 (0.11)	0.80
Never misused substances	PVH Fazekas score		0 (Reference)	

DWMH = Deep white matter hyperintensities, GMV = Grey matter volume, PVH = Periventricular hyperintensities, S.E = Standard error, WMV = White matter volume

4.4 Conclusion

The discussion of the results of the PREVENT Dementia sMRI data is presented below. The potential mechanisms and clinical and public health implications are presented in chapter 5.

4.4.1 Main findings

The findings that were significant across all the models (unadjusted and adjusted) from the analysis of the relationship between lifestyle and midlife sMRI outcomes are shown in Table 35.

Table 35: Summary of the results from the linear regression models of the relationship between lifestyle factors in midlife as expressed on structural MRI.

Lifestyle Factor	Structural MRI Outcome	Model	β coefficient (S.E)	p
Physical training score – medium	Hippocampal grey matter volume	Unadjusted model	-0.07 (0.03)	0.02*
		Adjusted (Model 1)	-0.07 (0.03)	0.03*
		Adjusted (Model 2)	-0.07 (0.03)	0.02 *
Physical training score – low	PVH Fazekas score	Unadjusted model	0.45 (0.13)	<0.01*
		Adjusted (Model 1)	0.44 (0.12)	<0.01*
		Adjusted (Model 2)	0.45 (0.13)	<0.01*
Smoker	GMV	Unadjusted model	-10.21 (3.19)	<0.01*
		Adjusted (Model 1)	-10.29 (3.19)	<0.01*
		Adjusted (Model 2)	-9.77 (3.75)	0.01*

GMV = Grey matter volume, PVH = Periventricular hyperintensities, S.E = Standard error

***=P ≤0.05**

Physical Training

The results from the unadjusted model, adjusted model 1 and adjusted model 2 show that compared to a high physical training score, a medium physical training score is associated with a smaller hippocampal grey matter volume. The hippocampus is located in the medial temporal lobe and the hippocampus as expressed on sMRI is shown in Figure 13.The hippocampus has an important role in the consolidation of information from short-term memory to long-term memory and in spatial memory (173).

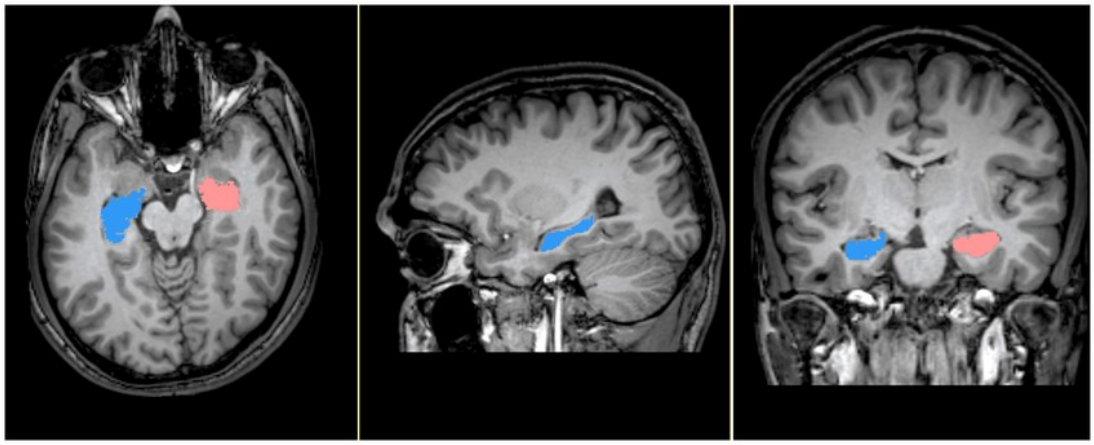

Figure 13: Hippocampus as expressed on sMRI in axial, sagittal and coronal sections (174).

Compared to a high physical training score, a low physical training score is associated with an increased PVH Fazekas score (158). The PVH Fazekas score is used to quantify the amount of white matter hyperintense lesions, these are usually due to chronic small vessel ischaemia (158). The PVH Fazekas scoring system is shown in Figure 14. A score of zero corresponds to an absence of white matter hyperintense lesions. A score of one, corresponds to pencil-thin lining around ventricles, two corresponds to a

smooth halo around ventricles (6-10mm regular margins) and three corresponds to an irregular halo > 10mm. White matter lesions, especially in the periventricular region are associated with an increased risk of dementia (175).

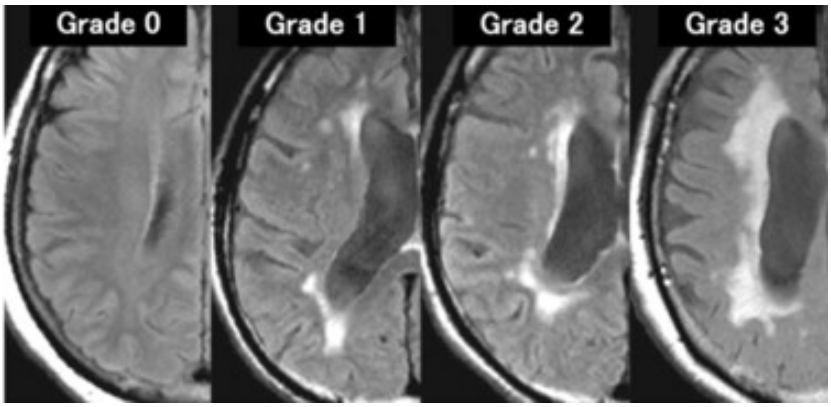

Figure 14: PVH Fazekas scoring system (176).

Smoking

The results from unadjusted model, adjusted model 1 and adjusted model 2 show that compared to being a non-smoker, being a smoker is associated with a reduction in GMV. Before symptom onset in Alzheimer's dementia, grey matter volume loss is detectable in specific regions, namely the anterior hippocampus and amygdala (177).

Additional findings

The results from the unadjusted model show that compared to being a non-smoker, being a smoker is associated with increased WMV. The results from adjusted model 1 show that compared to being a non-substance misuser, being a current or ex-substance misuser is associated with a reduction in GMV.

Linear regression model covariates

Historically, a combination of structural and functional brain imaging has been used for the purpose of analysing functional activity in *a priori* defined brain region. The sMRI image is used as a reference for locating regions of interest for fMRI analysis. However, a central assumption of systems neuroscience is that the structure of the brain is related to functional connectivity (178). The findings of Segall et al. (179) support this hypothesis. A cohort of 603 healthy participants underwent structural and functional scanning. Spatial independent component analysis was applied separately to both the structural MRI data and the resting state fMRI data. The results showed a correspondence between a single structural component and several resting-state functional components. Therefore the covariates identified in the systematic review of the relationship between lifestyle and neurodegeneration in midlife as expressed on fMRI have been used in the analysis of the PREVENT Dementia cohort (119) sMRI data.

Comparison with other literature

The main findings of this data analysis are supported by previous studies assessing the relationship between lifestyle factors and neurodegeneration as expressed on sMRI. In a randomised control trial of 120 late life adults, Erickson et al. (180) demonstrated that moderate intensity exercise on three days per week for one year increased the size of the anterior hippocampus.

Exercise training increased hippocampal volume by 2%. In the control group, Hippocampal volume declined by 1.4%, suggesting that moderate

intensity exercise protects against hippocampal volume loss. In a longitudinal cohort study of 299 late life adults, Erickson et al. (181) demonstrated that high volume low intensity exercise - walking, is associated with increased grey matter volume. In the study, 299 adults (mean age 78 years), were assessed for their level of physical activity. This was quantified by the number of blocks walked over one week. Walking amounts ranged from 0 to 300 blocks (mean 56.3; SD 69.7). After splitting the participants into quartiles (Q1, Q2, Q3, Q4) based on the number of blocks walked, the study found that GMV in the highest quartile (Q4) was greater than GMV in the other three quartiles (p <0.05).

Additionally, in a cross-sectional study of 37 alcohol dependent individuals in midlife, Gazdzinski et al. (182) demonstrated that chronic cigarette smoking is associated with reduced parietal and temporal grey matter volume and increased temporal white matter volume ($p < 0.05$). In the study, it was hypothesised that chronic cigarette smoking had an independent effect on regional GMV, perhaps reflecting neurotoxicity and subclinical oedematous processes associated with the direct and indirect effects of the many cytotoxic compounds found in cigarette smoke.

4.4.2 Strengths and limitations

There are important limitations in this analysis of the relationship between lifestyle and neurodegeneration in midlife as expressed on sMRI. The analysis is based on the findings of the systematic review of the relationship between lifestyle factors and neurodegeneration in midlife as expressed on fMRI. The linear regression models could be further refined if

they were based on a systematic review of the relationship between lifestyle factors and neurodegeneration in midlife as expressed on sMRI. Additionally, it is worth noting that although the linear regression models show a relationship between lifestyle factors and neurodegeneration in midlife as expressed on sMRI, causation cannot be inferred from the PREVENT Dementia cohort (119) cross-sectional data. Furthermore low power may have contributed to a reduced chance of detecting a true effect in the statistical analysis (169). A strength of this analysis is the large number of participants for whom sMRI data has been collected and assessed.

4.4.3 Summary

This exploratory analysis shows that multiple lifestyle factors in midlife are associated with neurodegeneration as expressed on sMRI. This analysis represents an early step in understanding the interplay between lifestyle factors and neurodegeneration in midlife as expressed on sMRI.

4.4.4 Implications of research

Although a number of associations were demonstrated by this data analysis, results to support all the hypotheses outlined at the start of the chapter were not ascertained. This may be due to low power in the statistical analysis. Looking to the future, this research could be expanded to look at how the changes in various biomarkers of lifestyle and neurodegeneration including ASL CBF and sMRI are related to each other. This was beyond the scope of this data analysis as the regions chosen for the ASL CBF analysis were based on the systematic review (chapter 2) and the regions assessed for the sMRI analysis were based on available measures from the PREVENT

Dementia cohort. Subsequently there was not an overlap in the regions available for both ASL CBF and sMRI analysis.

5 Conclusion

Chapter abstract

The research presented in this thesis, namely the systematic review (chapter 2), analysis of the PREVENT Dementia cohort ASL data (chapter 3) and sMRI data analysis (chapter 4) have led to important findings on the relationship between lifestyle and neurodegeneration in midlife. The systematic review identified five lifestyle factors associated with neurodegeneration in midlife as expressed on fMRI. The analysis of the PREVENT Dementia cohort ASL data has shown that that compared to being a non-substance misuser, being a current or ex-substance misuser is associated with a reduced ACC CBF. Also, compared to being a moderate alcohol drinker, being a non-drinker is associated with a reduction in ACC CBF. The ACC is thought to have an important role in ageing by mediating resistance of memory circuits to the deleterious processes of ageing. There was no significant association between physical training and smoking with CBF. Additionally, the sMRI linear regression analysis has identified multiple associations between lifestyle and neurodegeneration in midlife. The findings from this thesis could guide future analyses of PREVENT Dementia cohort data and test the hypothesis that substance misuse causes neurodegeneration in the ACC. Critically, causation cannot be inferred from any of the findings from this thesis, however the findings could be shared with the public through the NHS Health Check in England and the Keep Well

Programme in Scotland, to help make people aware of the relationship between lifestyle and brain health in midlife.

5.1 Key findings from my thesis on the relationship between lifestyle and neurodegeneration in midlife as expressed on ASL and sMRI

Systematic review

This systematic review of the relationship between lifestyle and neurodegeneration in midlife as expressed on fMRI, identified seven lifestyle factors - physical training, cognitive training, fasting, substance misuse, alcohol, smoking and excessive internet use, associated with brain health in midlife. Cognitive training and physical training appear to be associated with a neuroprotective effect, whereas alcohol misuse, smoking and substance misuse appear to be associated with neurodegeneration. Further research is required into the effects of excessive internet use and fasting.

Analysis of PREVENT Dementia cohort ASL data

The analysis of the relationship between lifestyle and neurodegeneration as expressed on ASL in midlife was undertaken using data from the PREVENT Dementia cohort (119). The results from the unadjusted model, adjusted model 1 and adjusted model 2 show that compared to being a non-substance misuser, being a current or ex-substance misuser is associated with a reduced ACC CBF. Additionally, the results from model 2 shows that compared to being a moderate alcohol drinker, being a non-drinker is associated with a reduction in ACC CBF. No

association was found between physical training and smoking with mean cortex CBF, ACC CBF and hippocampal CBF.

Structural magnetic resonance imaging analysis

In the analysis of the relationship between lifestyle and neurodegeneration as expressed on sMRI, the results from the unadjusted model and adjusted models show that compared to a high physical training score, a medium physical training score is associated with a reduced hippocampal grey matter volume; compared to a high physical training score, a low physical training score is associated with an increased PVH Fazekas score (158) and compared to being a non-smoker, being a smoker is associated with a reduced GMV. Additionally, on the unadjusted model, compared to being a non-smoker, being a smoker is associated with increased WMV and on adjusted model 1, compared to being a non-substance misuser, being a current or ex-substance misuser is associated with a reduction in GMV.

5.2 What this thesis adds to the field of dementia prevention

The information retrieved by the systematic review has been used as a basis for the analysis of lifestyle and neuroimaging data in the PREVENT Dementia cohort (119). The systematic review guided the choice of lifestyle factors, conceptualization of lifestyle factors and choice of covariates for the linear regression models. The systematic review could be used as a basis for further data analysis, including the two year follow-up data from the PREVENT Dementia cohort (119). The systematic review could help guide

the choice of lifestyle interventions and biomarkers in future dementia prevention studies. Furthermore, the systematic review has been published (183). Through publication, the systematic review can more broadly contribute to research in the fields of lifestyle, neurodegeneration and fMRI. For example, the systematic review has been cited by Huang et al. in their study of 'The correlation of asymmetrical functional connectivity with cognition and reperfusion in carotid stenosis patients (184)'. It has also been cited by Hadar et al. in their review of peripheral transcriptomic biomarkers for early detection of sporadic Alzheimer's dementia (185).

The *a priori* hypothesis that substance misuse is associated with neurodegeneration in midlife as expressed on ASL, was based on the findings of the systematic review. The systematic review captured studies that showed that heroin, cocaine and polysubstance use are associated with reduced activity in the ACC in midlife as expressed on fMRI (94, 102, 107, 115). The analysis of the PREVENT Dementia cohort data supported the *a priori* hypothesis, by demonstrating that compared to non-substance misusers, being a substance misuser is associated with a reduction in the ACC CBF in midlife as expressed on ASL. This is likely to represent early neurodegenerative changes in the ACC. To my knowledge, this is a unique finding in the literature and opens up a gateway for further research to be undertaken into the relationship between substance misuse and neurodegeneration as expressed on ASL. There is scope for further research into the effect of different substances, duration of use and method of administration of drug e.g. oral, intravenous and smoking on brain health.

However, it is worth considering that the association between being a substance misuser and a reduction in the ACC CBF may represent a type I error, as no association was found between smoking and physical training with mean cortex CBF, ACC CBF and hippocampal CBF.

The PREVENT Dementia cohort (119) analysis also showed that compared to having moderate alcohol consumption, being a non-drinker is associated with a reduced ACC CBF. This finding adds to the findings from a study by Kim et al. (96) which assessed the relationship between alcohol and neurodegeneration in midlife as expressed on fMRI. In this study, Kim et al. (96) assessed ReHo in outpatients with alcohol use disorder. The ReHo value indicates the local connectivity of the temporal correlations between a given voxel and adjacent voxels (186, 187). The study results showed a significant reduction in ReHo in the ACC of patients with AUD. This may represent abnormal ReHo, which is thought to be related to pathological changes (188). Together, these findings build on literature which suggests that moderate alcohol consumption may be beneficial for brain health and excessive consumption may have a detrimental effect on brain health (35).

The findings from the analysis of the relationship between lifestyle and neurodegeneration as expressed on sMRI found multiple associations between lifestyle factors and fMRI outcomes. This represents an important initial step in unravelling the relationship lifestyle factors and neurodegeneration in midlife as expressed on sMRI.

5.3 Potential mechanisms

5.3.1 Arterial spin labelling findings

The anterior cingulate cortex and substance misuse

The anterior cingulate cortex resides in the medial wall of the cerebral hemispheres, immediately above the corpus callosum and is shown in Figure 15. The cingulate cortex can be broadly divided into the anterior cingulate, mid cingulate and posterior cingulate. As a whole, the cingulate cortex integrates input related to motivation, evaluation of error, cognition and emotion (189). More specifically, the ACC is involved in a variety of cognitive and emotional tasks including pain processing, visuospatial processing and memory retrieval (189).

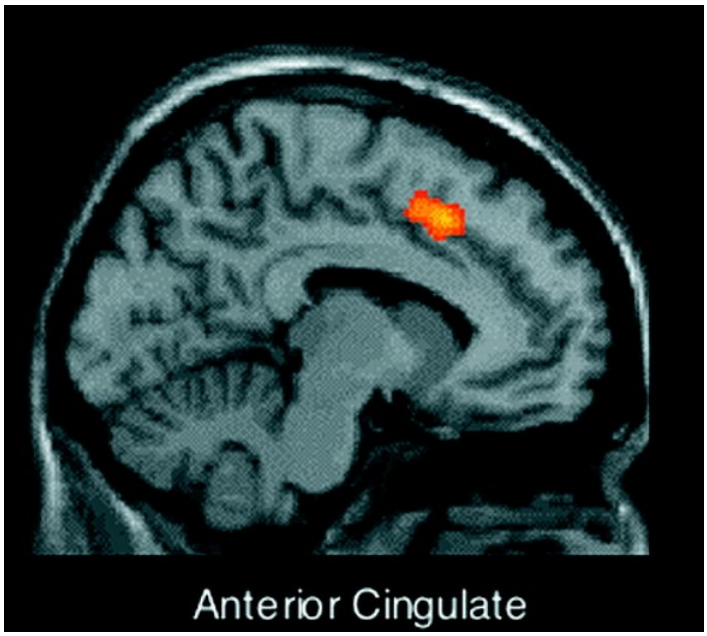

Figure 15: Anterior cingulate cortex as expressed on fMRI (190).

Studies identified by the systematic review of the relationship between lifestyle and neurodegeneration in midlife as expressed on fMRI have shown an association between substance misuse and reduced activity in the ACC

(94, 102, 107, 115). These studies are described in Chapter 2 and hypothesize that the direction of the association, is that reduced activity in the ACC results in individuals being more likely to misuse substances. This direction of association is supported by research by Lee et al. that found decreased activity in dorsal ACC was associated with impulsive characteristics of heroin users (191). The dorsal ACC is the cognitive subdivision of the ACC and is responsible for inhibition controlling and conflict monitoring. The studies do not put forward a hypothesis for why there might be reduced activity in the ACC.

A potential mechanism for the reduction in ACC CBF associated with substance misuse identified in the PREVENT Dementia cohort data (119), is related to overstimulation of glutamate receptors. Substance misuse can result in over activation of several neurotransmitter pathways, including the glutamatergic pathway (192). Overstimulation of glutamate receptors results in an uncontrolled increase of calcium that causes damage to proteins, nucleic acids, lipids and the cell membrane. This subsequently leads to apoptotic or necrotic neuronal death (192). The full pathway for the cellular and molecular alterations induced by substance misuse that result in apoptotic or necrotic neuronal death is shown in Figure 16 (192).

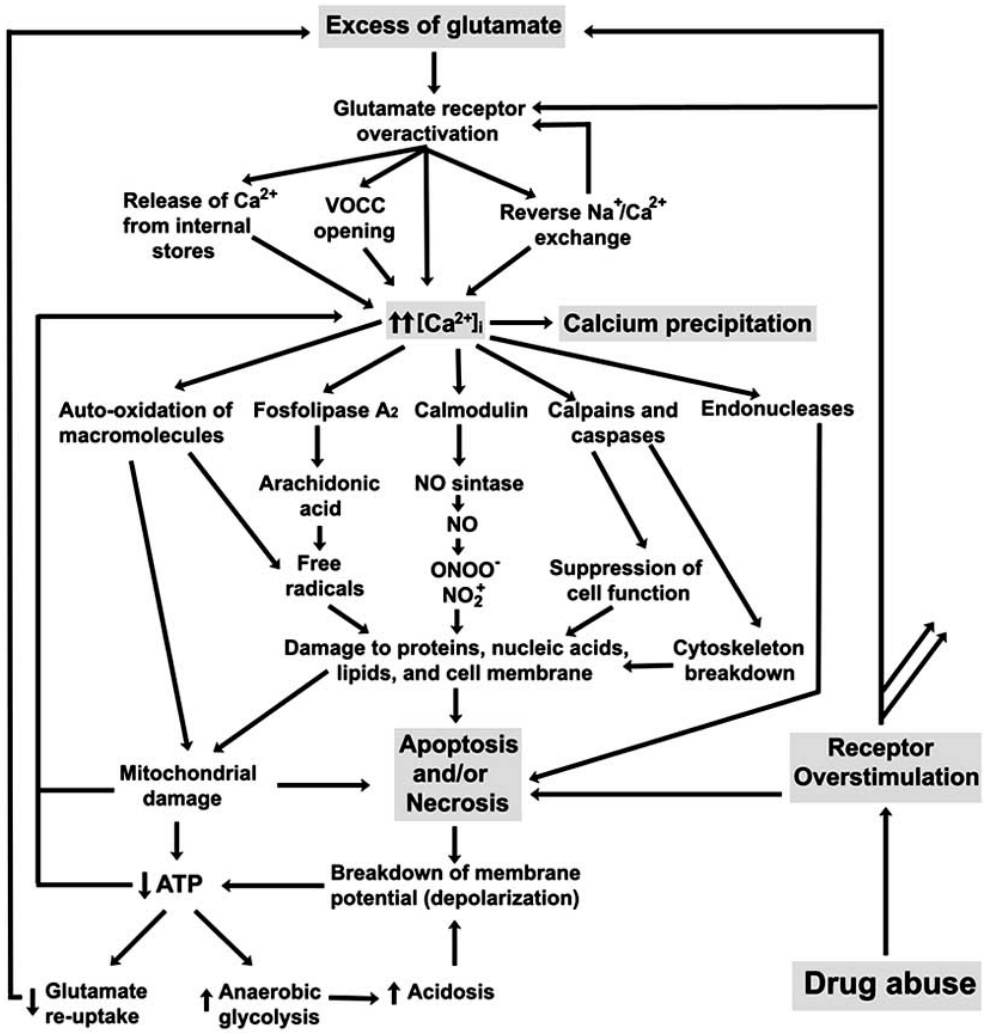

Figure 16: Cellular and molecular alterations induced by substance misuse resulting in an uncontrolled increase of calcium and apoptotic or necrotic neuronal death (192).

A hypothesis, that could be tested by future analyses of the PREVENT Dementia cohort follow-up data, is that the direction of association is that substance misuse causes neurodegeneration in the ACC. It is important to better understand the direction of association, as the ACC is thought to play a key role in brain health. Harrison et al. demonstrated that prominence of the ACC as expressed on sMRI has been linked to successful cognitive ageing (193). In the study, cognitive 'SuperAgers' were assessed for cortical brain volume loss using sMRI. 'SuperAgers' were defined as individuals over

the age of 80 years old with episodic memory performance at least as good as normative values for 50 to 65 year olds. Cortical morphometry of the 'SuperAgers' were compared to two cognitively normal cohorts: age matched elderly and 50 to 65 year olds. The 'SuperAgers' cerebral cortex was significantly thicker than their healthy age matched peers and displayed no atrophy compared to the 50 to 65 year old healthy group. Unexpectedly, a region of left anterior cingulate cortex was significantly thicker in the 'SuperAgers' than in both elderly and middle aged controls. The authors hypothesised that the ACC may mediate resistance of memory circuits to the deleterious processes of ageing (193).

5.3.2 Structural MRI findings that were statistically significant across all models (unadjusted and adjusted)

Physical training and hippocampal volume

Cell proliferation or increased dendritic branching in response to exercise may explain the association between increased hippocampal volume and increased physical training in midlife. This hippocampus is rich in brain derived neurotrophic factor (BDNF). BDNF is a mediator of neurogenesis and contributes to dendritic expansion and cell proliferation (194, 195). Ramussen et al. demonstrated that BDNF levels increase with exercise (196). In the study, eight volunteers rowed for four hours while simultaneous blood samples were obtained. The internal jugular venous BDNF concentration increased from 442 ± 272 pg ml^{-1} at rest to 1172 ± 968

pg ml^{-1} after four hours of exercise ($P < 0.05$) and returned to the resting level after one hour of recovery ($P < 0.05$).

Physical training and PVH Fazekas score

Increased physical training may help manage an individual's vascular risk factors and subsequently reduce their Fazekas score (158) which is a measure of white matter lesions. Multiple studies have shown that physical training decreases vascular risk factors including hypercholesterolemia and hypertension. In a review of the relationship between cholesterol and exercise, Pedersen et al., citing 13 meta-analyses, reported improvements in the lipid profile following exercise (197). Interventional studies by Rodríguez-Rodríguez et.al and Atkinson et al. have demonstrated that increased physical activity reduces blood pressure in hypertensive and normotensive individuals independently from weight loss (198, 199).

Smoking and grey matter volume

Smoking contributing to increased atherosclerosis and causing a direct effect on brain morphology could account for the relationship between smoking and GMV in midlife as expressed on sMRI. There is evidence that smoking is associated with an increased risk of atherosclerosis. This was demonstrated in a study by Howard et al., where 10,914 participants from the Atherosclerosis Risk in Communities (ARIC) were assessed for change in atherosclerosis from baseline to the 3-year follow-up (200). Change in atherosclerosis was measured using intimal-medial thickness of the carotid artery. Relative to non-smokers and after adjustment for demographic

characteristics, cardiovascular risk factors, and lifestyle variables, smoking was associated with a 50% increase in the progression of atherosclerosis. The mean progression rate over 3 years was 43.0 μm for current and 28.7 μm for non-smokers. Decreased cerebral blood flow due to atherosclerosis could account for a reduction in grey matter volume (201). Additionally, smoking which results in a toxic and carcinogenic mixture of more than 5,000 chemicals, has been hypothesized to have direct effects on brain morphology (202). This could also could account for the reduction in grey matter volume.

5.4 Future research

There is scope to expand the analysis undertaken in this thesis as further data becomes available from the PREVENT Dementia study (119). Recently cerebral microbleed data has become available for analysis. Cerebral microbleeds are downstream markers of brain damage caused by both vascular and amyloid pathological mechanisms. In the Rotterdam Study, the presence, number, and location of microbleeds at baseline (2005–2011) on sMRI scans in 4,841 participants aged over 45 years old were assessed. Participants were followed up for incident dementia throughout the study period until 2013. The study found that the presence of microbleeds were associated with an increased risk of dementia (HR 2.02, 95% CI 1.25 - 3.24), including Alzheimer's dementia (HR 2.10, 95% CI 1.21 - 3.64) (203).

In the future, a larger volume of PREVENT Dementia cohort (119) data patient data will become available thereby increasing the statistical power of future analysis. Data is currently being collected across five study

sites in the UK and Ireland. The study sites are at the Universities of Edinburgh, Oxford, Cambridge, Imperial College London and Trinity College Dublin.

An alternate approach that may be of benefit in the analysis of the PREVENT Dementia cohort (119) data is to form a composite risk score for brain health. This approach was adopted by the Caerphilly Prospective Study (CaPS). The CaPS is based on a cohort of men in a small town in South Wales UK. The men have been repeatedly questioned and examined for over 30 years. The study assessed the correlation between lifestyle behaviours and cognitive impairment in CaPS. The healthy lifestyles behaviours that were assessed were non-smoking, an acceptable BMI, a high fruit and vegetable intake, regular physical activity and low to moderate alcohol intake. The study found that the OR for men following four or five healthy behaviours was 0.36 (95% CI: 0.12 - 1.09; P = <0.01) for cognitive impairment, and 0.36 (95% CI: 0.07 - 1.99; P = 0.02) for dementia (68). Using the PREVENT Dementia cohort, a similar composite risk score could be formed based on the lifestyle factors used in the analysis undertaken in chapter 3, namely smoking, alcohol, physical training and substance misuse.

5.5. Clinical and public health implications

There are 850,000 people with dementia in the UK (12). This figure is estimated to pass one million by 2025 and exceed two million by 2050. The annual cost of dementia to society in the UK is estimated at £26.3 billion. This is higher than the cost of cancer, heart disease, or stroke (12). Given the

significant burden of dementia on society, ongoing research to reduce the incidence of dementia is essential. In the NHS 5 year Forward View it was noted that we can reduce the incidence of dementia through tackling lifestyle risks (204). Research in my thesis has shown an association between lifestyle and neurodegeneration in midlife as expressed on ASL and sMRI. It is worth reiterating that this cross sectional analysis cannot prove causality, however it can help generate causal hypothesis. Based on my thesis, I propose that lifestyle modification could potentially alter neurodegeneration in midlife and thereby reduce an individual's risk of late life dementia. Long term randomised controlled trials are needed to test this hypothesis. If future trials show sufficient evidence to support this hypothesis, the NHS Health Check in England (205) and the Keep Well Programme in Scotland (206) would provide valuable opportunities for GPs and other healthcare professionals to offer advice to promote lifestyle interventions to optimise brain health. The NHS Health Check is a check-up for individuals in England aged 40 to 74 years old. It is designed to identify early signs of stroke, kidney disease, heart disease, type 2 diabetes or dementia and designed to identify ways to lower the risks of these conditions (205). The Keep Well Programme in Scotland aims to increase the rate of health improvement in individuals' aged 40 to 64 in deprived communities by enhancing primary care services to deliver anticipatory care. The checks include screening for cardiovascular disease and its main risk factors, such as high blood pressure, cholesterol, smoking, diet as well as discussions around wider life circumstances such as employment and literacy (206). The discussions could be extended to include

discussion around lifestyle interventions in midlife. Clinical Commissioning Groups (CCG) can play a key role in promoting dementia risk reduction. CCG can achieve this by using Public Health England's dementia profile tool which can identify local risk factors for dementia such as smoking prevalence, physical inactivity, excess weight and alcohol-related hospital admissions (207). This will help to prioritise efforts to reduce such risk factors. Furthermore, health and social care providers, in addition to public and third sector providers, such as local authorities, leisure services and emergency services could use routine appointments to identify people at risk of dementia, promote healthy behaviours and give advice on how to reduce the risk factors for dementia whenever the opportunity arises.

5.6. Final Conclusions

The clinical and economic need to prevent dementia and the failure of medication breakthroughs to treat Alzheimer's dementia have led to considerable research in the field of optimising brain health. The evidence on the relationship between lifestyle and brain health has now reached a point that it can no longer remain simply an exercise in academic discussion. The evidence should be shared with the public. The public should know that there is a relationship between lifestyle and brain health. Looking to the future, this thesis forms an exciting platform for further research in the field of lifestyle and neurodegeneration as expressed on neuroimaging to build upon and continue our drive to reduce the worldwide incidence of dementia.

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Appendix A

Alzheimer's & Dementia: Translational Research & Clinical Interventions 4 (2018) 182-194

Review Article

Lifestyle and neurodegeneration in midlife as expressed on functional magnetic resonance imaging: A systematic review

Hinesh Topiwala[a,*], Graciela Muniz Terrera[a], Lucy Stirland[a], Kathryn Saunderson[a], Tom C. Russ[a,b], Marshall F. Dozier[c], Craig W. Ritchie[a]

[a]Centre for Dementia Prevention, Centre for Clinical Brain Sciences, University of Edinburgh, Edinburgh, UK
[b]Alzheimer Scotland Dementia Research Centre, University of Edinburgh, Edinburgh, UK
[c]Information Services, University of Edinburgh, Edinburgh, UK

Abstract

Introduction: Lifestyle factors may influence brain health in midlife. Functional magnetic resonance imaging is a widely used tool to investigate early changes in brain health, including neurodegeneration. In this systematic review, we evaluate the relationship between lifestyle factors and neurodegeneration in midlife, as expressed using functional magnetic resonance imaging.
Methods: We searched MEDLINE, EMBASE, and PsycINFO combining subject headings and free text terms adapted for each database. Articles were screened, and their quality was assessed independently by two reviewers before final inclusion in the review.
Results: We screened 4116 studies and included 29 in the review. Seven lifestyle factors, such as alcohol, cognitive training, excessive internet use, fasting, physical training, smoking, and substance misuse, were identified in this review.
Discussion: Cognitive and physical trainings appear to be associated with a neuroprotective effect, whereas alcohol misuse, smoking, and substance misuse appear to be associated with neurodegeneration. Further research is required into the effects of excessive internet use and fasting.

Keywords: Neurodegeneration; Neuroprotection; Magnetic resonance imaging; Life Style; Middle aged

1. Background

There is increasing recognition that neurodegenerative diseases, which manifest clinically as dementia in later life, have their origins in midlife, or even earlier [1]. Recent research on cognitive, neuroimaging, and biological markers suggest that changes in several parameters may well precede overt clinical symptoms by not just many years, but decades [2]. Functional magnetic resonance imaging (fMRI) offers considerable promise as a marker for neurodegenerative disease. It could also be of value in monitoring disease progression and response to interventions [3] such as lifestyle modification. In midlife, lifestyle modification could potentially alter neurodegenerative disease progression and thereby reduce an individual's risk of dementia in later life [4]. If this were the case, it is critical to identify which potentially modifiable lifestyle factors are associated with neurodegeneration in midlife. Therefore, in the absence of any previous systematic reviews, we evaluate the relationship between lifestyle factors and neurodegeneration in midlife as expressed on fMRI in the published literature.

2. Methods

2.1. Identification of studies

MEDLINE, EMBASE, and PsycINFO were searched via the OVID platform on 5th December 2016. MEDLINE was

Conflicts of interest: The authors have declared that no conflict of interest exists.

*Corresponding author. Tel.: +44 (0)131 537 6000; Fax: +44 (0)131 242 7184.
E-mail address: htopiwala1@nhs.net

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searched from 1946 to November 2016, EMBASE from 1980 to November 2016, PsycINFO from 1806 to November 2016. There were no limits on language or publication dates. A specific search was constructed for each database using subject headings and free text terms, and these can be found in the Supplementary Material. The search terms covered the areas of neuroimaging, lifestyle, and regional changes in cerebral metabolism or blood flow, blood volume, or oxygenation.

The systematic review aimed to include all published studies that assessed the relationship between lifestyle factors and neurodegeneration, neuroprotection, or both as expressed on fMRI in midlife. A study was defined as having assessed individuals in midlife if it included individuals aged 40–59 years or if two standard deviations around the mean fell within the 40–59 years age range. Neurodegeneration was defined as any pathological condition primarily affecting neurons [5]. Neuroprotection was considered to be an effect that may result in salvage, recovery, or regeneration of the nervous system, its cells, structure, and function [6]. The exposure in the review was lifestyle factors, as defined by the World Health Organization: "Lifestyle is a way of living based on identifiable patterns of behavior, which are determined by the interplay between an individual's personal characteristics, social interactions, and socioeconomic and environmental living conditions" [7]. The outcome in the systematic review was the numerical outcome measures derived from the fMRI scan. fMRI is a brain imaging technique to capture regional changes in cerebral metabolism or in blood flow, volume, or oxygenation in response to task activation or during rest [8]. The systematic review aimed to include studies using both resting-state and task-based fMRI experimental protocols and studies assessing the general population and those conducted in a general medical setting. There were no limits by language or publication date.

2.2. Eligibility

The inclusion/exclusion criteria for the systematic were as follows:

Inclusion criteria
i. Original human research study.
ii. Population includes a lifestyle factor in midlife.
iii. Study includes an fMRI outcome in midlife.

Exclusion criteria
i. Not an original human research study.
ii. Study population has a diagnosis of dementia either in general or based on specific subtypes classified using standard diagnostic criteria, for example, the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association [9].
iii. Study population does not include a lifestyle factor in midlife.
iv. Study population does not include individuals in midlife.
v. The aim of the study is not to look at the effect of a lifestyle factor on fMRI outcome.
vi. fMRI outcome is not a proxy for neurodegeneration or neuroprotection.

2.3. Study selection and data collection

The titles and abstracts of all articles identified by the search were screened independently by two reviewers against the inclusion and exclusion criteria with any disagreements in the final lists of included studies resolved by discussion. Potentially relevant articles were then retrieved and examined against the inclusion and exclusion criteria. Differences between reviewers' selections were again resolved by discussion. Data were extracted from included articles by one reviewer on the number of study participants, mean age, standard deviation and age range of participants, study methodology and design, and the key findings from the study related to this systematic review.

2.4. Quality of evidence

The quality of studies was assessed using a modified version of the Effective Public Health Practice Project Quality Assessment Tool [10] tailored to the literature being assessed in this review. This tool has been judged suitable for use in a systematic review [11] and forms a global quality rating for a paper based on six assessment criteria: selection bias, study design, confounders, blinding, data collection method, and withdrawals and dropouts.

2.5. Protocol and registration

The systematic review protocol was registered on the PROSPERO International Prospective Register of Systematic Reviews, registration number CRD42016045237 (https://www.crd.york.ac.uk/PROSPERO/).

3. Results

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses diagram (Fig. 1) for the screening and selection of studies shows that 4116 records were identified through database searches. Following de-duplication and title and abstract screening, 255 full-text articles were assessed for eligibility. After excluding 226 articles for the reasons outlined in Fig. 1, a total of 29 articles were included in the systematic review. Table 1 gives a summary overview of the 29 articles included in the systematic review, arranged by lifestyle factors. Table 2 then summarizes the key findings from the 29 individual articles.

3.1. Alcohol

Of the six studies looking at the effect of alcohol as expressed on fMRI, three used a task-based fMRI protocol, and three studies used a resting-state protocol. Of the

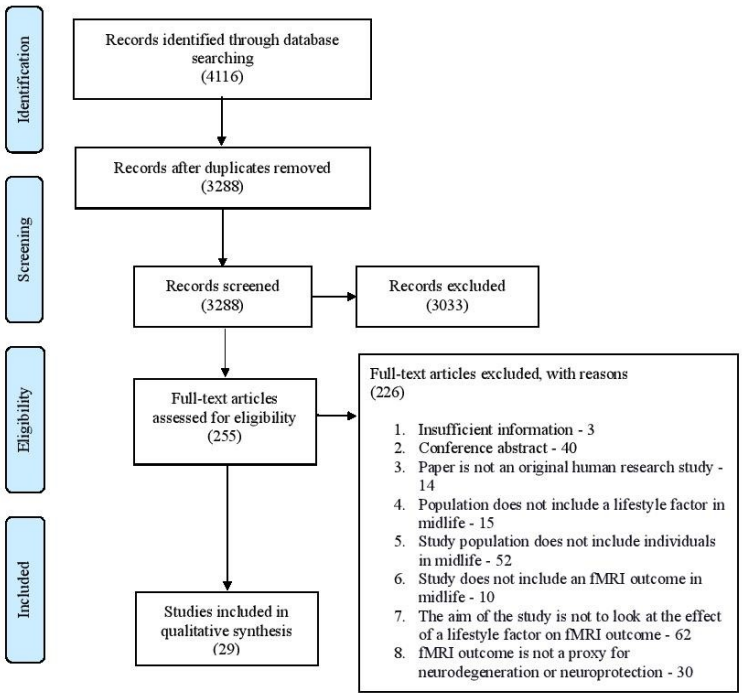

Fig. 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses diagram showing the selection of studies from search to inclusion.

resting-state studies, Gazdzinski et al. [19] demonstrated the evidence that concurrent alcohol dependence and chronic cigarette smoking are associated with regional gray matter hypoperfusion. Kim et al. [27] showed increased regional homogeneity (ReHo) in the posterior cingulate cortex (PCC) executive control, basal ganglia, and primary visual networks. Weiland et al. [39] found significantly lower network connectivity strength than controls in the left executive control, basal ganglia, and primary visual networks. For the task-based studies, Hermann et al. [41] described significantly lower blood-oxygen-level dependent signal in an extended bilateral occipital area as compared with healthy controls during a visual and acoustic simulation task. Rogers et al. [36] described a pattern in recently abstinent alcoholic patients of specific deficits in functional connectivity and recruitment of additional brain regions for the performance of a simple finger-tapping task. Sullivan et al. [37] demonstrated that alcoholics have selective differences from control subjects in the cerebral blood flow (CBF) pattern in the anterior precuneus and CBF level in the insula, a hub of the salience network.

Table 1
Summary of evidence for each lifestyle factor identified in the systematic review

Lifestyle factor	Number of studies	Quality assessment rating of studies	Overall effect—neurodegenerative or neuroprotective
Physical training	7	Moderate: 2 studies Strong: 5 studies	Neuroprotective
Cognitive training	3	Strong: 3 studies	Neuroprotective
Fasting	1	Weak: 1 study	Neuroprotective
Substance misuse	12	Weak: 4 studies Moderate: 4 studies Strong: 4 studies	Neurodegenerative
Alcohol	6	Weak: 3 studies Moderate: 3 studies	Neurodegenerative
Smoking	3	Weak: 1 study Moderate: 1 study Strong: 1 study	Neurodegenerative
Excessive internet use	1	Moderate: 1 study	Neurodegenerative

3.2. Cognitive training

Three studies looked at the effect of cognitive training as expressed on fMRI. Chapman et al. [16] examined changes

Table 2
Individual studies reporting on the relationship between lifestyle factors and neurodegeneration in midlife as expressed on fMRI

Study	Lifestyle factor	N	Mean age (SD)/Range	Methodology/ design	Findings	Quality assessment rating	Neurodegenerative or neuroprotective effect
Bednarski et al. [12]	Substance misuse	50 (cocaine dependence 23/healthy controls 27).	Cocaine dependence: 36.2 (6.3)/NA. Healthy controls: 34.9 (6.5)/NA.	Study type: cross-sectional study. fMRI type: task-based. Statistical method: one sample and two-sample t-tests.	Cocaine dependence is associated with dysfunction of the default mode network.	Moderate	Neurodegenerative
Belaich et al. [13]	Fasting	6 males.	41 (NA)/34 – 48.	Study type: cross-sectional study. fMRI type: task-based. Statistical method: ANOVA test.	There is a significant difference between maximal BOLD-fMRI signal before and during fasting.	Weak	Neuroprotective
Castelluccio et al. [14]	Substance misuse	94 (cocaine users 30/former users 29/healthy controls 35).	Cocaine users: 37.6 (7.3)/ 21–45. Former users: 40.3 (6.4)/22–50. Healthy controls: 35.8 (10)/21–58.	Study type: cross-sectional study. fMRI type: task-based. Statistical method: regression analysis.	The cocaine user groups exhibited significantly increased BOLD activity relative to healthy controls in several a priori regions of interest.	Weak	Neurodegenerative
Chapman et al. [15]	Physical training	37 (physical training 18/ control 19).	64 (3.9)/57–75.	Study type: controlled clinical trial. fMRI type: resting-state. Statistical method: general statistical linear model.	There is higher resting CBF in the anterior cingulate region in the physical training group compared to the control group.	Strong	Neuroprotective
Chapman et al. [16]	Cognitive training	37 (cognitive training 18/ control 19).	62.9 (3.6)/56–71.	Study type: controlled clinical trial. fMRI type: resting-state. Statistical method: general statistical linear model.	There was increased global and regional CBF particularly in the default mode network and the central executive network in the cognitive training group.	Strong	Neuroprotective
Chapman et al. [17]	Physical or cognitive training	36 (physical training 18/ cognitive training 18).	Physical training: 64 (4.3)/ 56–75. Cognitive training: 61.8 (3.3)/ 56–75.	Study type: randomized control trial. fMRI type: resting-state. Statistical method: general statistical linear model.	There were multiple distinct changes on fMRI that suggest that aerobic exercise and cognitive reasoning training contribute differentially to brain health.	Strong	Neuroprotective

(*Continued*)

Table 2
Individual studies reporting on the relationship between lifestyle factors and neurodegeneration in midlife as expressed on fMRI (*Continued*)

Study	Lifestyle factor	N	Mean age (SD)/Range	Methodology/ design	Findings	Quality assessment rating	Neurodegenerative or neuroprotective effect
Durazzo et al. [18]	Smoking	61 (smokers 34/ nonsmokers 27).	Smokers: 47.3 (10.5)/NA. Nonsmokers: 47.3 (11.9)/NA.	Study type: cross-sectional study. fMRI type: resting-state. Statistical method: multivariate analysis of covariance.	Smokers showed significantly lower perfusion than nonsmokers in multiple brain regions including regions implicated in early Alzheimer's disease (cingulate, right isthmus of the cingulate, right supramarginal gyrus, and bilateral inferior parietal lobule).	Weak	Neurodegenerative
Gazdzinski et al. [19]	Alcohol and smoking	48 (nonsmoking light drinkers 19/nonsmoking alcoholics 10/smoking alcoholics 19).	Nonsmoking light drinkers: 47 (7.9)/26–66. Nonsmoking alcoholics: 50.9 (10)/26–66. Smoking alcoholics: 48 (9.9)/26–66.	Study type: cross-sectional study. fMRI type: resting-state. Statistical method: MANOVA; Wilks' λ.	Alcoholics, as a group, showed lower frontal gray matter perfusion and lower parietal gray matter perfusion than nonsmoking light drinkers. In smoking alcoholics, a higher number of cigarettes smoked per day was associated with lower perfusion.	Moderate	Neurodegenerative
Hart et al. [20]	Physical training	52 (ex-NFL players 26/healthy controls 26).	Ex-NFL players: 61.8 (NA)/ 41–79. Healthy controls: 60.1 (NA)/ 41–79.	Study type: cross-sectional study. fMRI type: resting-state. Statistical method: NA.	Altered CBF patterns in retired NFL players are concordant with brain regions associated with abnormal findings on neuropsychological testing.	Strong	Neurodegenerative
Hermann et al. [21]	Alcohol	18 (alcohol dependence 9/healthy volunteers 9).	Alcohol dependence: 40.2 (5.6)/NA. Healthy control: 41.8 (13.2)/NA.	Study type: cross-sectional study. fMRI type: task-based. Statistical method: general statistical linear model.	Alcohol-dependent patients showed a significantly lower BOLD signal in an extended bilateral occipital area as compared with healthy controls.	Weak	Neurodegenerative
Hotting et al. [22]	Physical and cognitive training	33 (cycling and spatial 8, cycling and perceptual 8, stretching and spatial 9, stretching and perceptual 8).	Cycling and spatial: 50.25 (4.2)/40–55. Cycling and perceptual: 49 (4.28)/40–55. Stretching and spatial: 50.22 (2.91)/ 40–55. Stretching and perceptual: 46 (3.89)/ 40–55.	Study type: controlled clinical trial. fMRI type: task-based. Statistical method: one and two-sample t-tests, a flexible factorial model and full factorial model.	Participants of the spatial training group showed lower activity than participants of the perceptual training group in a network of brain regions associated with spatial learning, including the hippocampus and parahippocampal gyrus.	Strong	Neuroprotective

(*Continued*)

Table 2
Individual studies reporting on the relationship between lifestyle factors and neurodegeneration in midlife as expressed on fMRI (*Continued*)

Study	Lifestyle factor	N	Mean age (SD)/Range	Methodology/ design	Findings	Quality assessment rating	Neurodegenerative or neuroprotective effect
Hu et al. [23]	Substance misuse	112 (cocaine users 56/healthy controls 56).	Cocaine users: 39.86 (6.71)/NA. Healthy controls: 38.70 (10.9)/ NA.	Study type: cross-sectional study. fMRI type: resting-state. Statistical method: ANOVA test.	Compared with healthy controls, the cocaine user group showed differing patterns of rsFC in multiple brain regions.	Moderate	Neurodegenerative
Ide et al. [24]	Substance misuse	163 (cocaine dependent 75/healthy controls 88).	Cocaine dependent: 39.9 (7.6)/NA. Healthy controls: 38.7 (10.9)/NA.	Study type: cross-sectional study. fMRI type: task-based. Statistical method: two-sample t-tests and multiple regression.	Compared with healthy controls, cocaine-dependent individuals showed decreased PSS and PSSI in multiple frontoparietal regions.	Moderate	Neurodegenerative
Jiang et al. [25]	Substance misuse	48 (chronic heroin users 24/normal controls 24).	Chronic heroin users: 35.67 (5.66)/NA. Normal controls: 35.38 (6.02)/NA.	Study type: cross-sectional study. fMRI type: resting-state. Statistical method: two-sample t-tests and a Pearson correlation analysis.	Compared with controls, heroin addicts had altered ALFF in multiple brain regions.	Strong	Neurodegenerative
Kelly et al. [26]	Substance misuse	49 (cocaine dependent 25/healthy comparisons 24).	Cocaine-dependent adults: 35.0 (8.8)/NA. Healthy comparisons: 35.1 (7.5)/NA.	Study type: cross-sectional study. fMRI type: resting-state. Statistical method: NA.	Reduced prefrontal interhemispheric rsFC in cocaine-dependent participants relative to control subjects and a cocaine dependence-related reduction in interhemispheric RSFC among nodes of the dorsal attention network.	Strong	Neurodegenerative
Kim et al. [27]	Excessive internet and alcohol use	45 (internet gaming disorder 16/alcohol use disorder 14/healthy controls 15).	Internet gaming disorder: 21.63 (5.92)/NA. Alcohol use disorder: 28.64 (5.92)/NA. Healthy controls: 25.40 (5.92)/NA.	Study type: cross-sectional study. fMRI type: resting-state. Statistical method: two-sample t-tests.	There were distinctive functional changes in the resting-state of patients with internet gaming disorder, in addition to common ReHo changes in the internet gaming disorder and alcohol use disorder group.	Moderate	Neurodegenerative
Lee et al. [28]	Substance misuse	23 (chronic cocaine abusers 13/healthy controls 10).	Chronic cocaine abusers: 38 (6)/28–45. Healthy controls: 36 (6)/27–44.	Study type: cross-sectional study. fMRI type: task-based. Statistical method: ANOVA, post hoc *t*-test, Pearson product moment correlations.	Chronic cocaine abusers showed significantly enhanced positive BOLD response to photic stimulation when compared with control subjects.	Weak	Neurodegenerative

(*Continued*)

Table 2
Individual studies reporting on the relationship between lifestyle factors and neurodegeneration in midlife as expressed on fMRI (*Continued*)

Study	Lifestyle factor	N	Mean age (SD)/Range	Methodology/ design	Findings	Quality assessment rating	Neurodegenerative or neuroprotective effect
Liu et al. [29]	Substance Misuse	26 (cocaine addict 13/ control subjects 13).	Cocaine-addicted subjects: 46.6 (6.9)/NA. Control subjects: 44.4 (6.0)/NA.	Study type: cross-sectional study. fMRI type: resting-state. Statistical method: Kolmogorov–Smirnov test, Mann–Whitney *U* test, linear regression.	In cocaine-addicted subjects, cerebral metabolic rate of oxygen, a surrogate marker of aggregated neural activity, was significantly lower than that in controls.	Weak	Neurodegeneration
MacIntosh et al. [30]	Physical Training	30 men with coronary artery disease.	65 (7)/55–80	Study type: cohort study. fMRI type: resting-state. Statistical method: linear regression analysis.	Perfusion was associated with fitness at baseline and with greater fitness gains with exercise.	Moderate	Neuroprotection
May et al. [31]	Substance misuse	42 (recently abstinent methamphetamine dependent 25/healthy controls 17).	Abstinent methamphetamine-dependent individuals: 38.84 (9.16)/NA. Healthy controls: 38.77 (9.40)/NA.	Study type: cross-sectional study. fMRI type: task-based. Statistical method: linear mixed effects analysis.	Methamphetamine-dependent individuals exhibited lower anterior insula, dorsal striatum, and thalamus activation than healthy controls, across anticipatory and stimulus-related processing.	Weak	Neurodegeneration
McFadden et al. [32]	Physical Training	12 overweight/ obese adults.	38.2 (9.5)/NA.	Study type: cohort study. fMRI type: resting-state. Statistical method: *t*- test.	A 6-month exercise intervention was associated with a reduction in default mode network activity in the precuneus.	Moderate	Neuroprotection
Mitchell et al. [33]	Substance misuse	32 (cocaine dependence 16/healthy controls 16).	Cocaine dependence: 39 (10.4)/NA. Healthy controls: 40 (7.4)/NA.	Study type: cross-sectional study. fMRI type: task-based. Statistical method: two-sample t-tests.	Cocaine-dependent patients displayed less overall intrinsic connectivity compared with healthy controls.	Strong	Neurodegeneration
Mon et al. [34]	Smoking	69 (nonsmoking light drinker 28, nonsmoking alcoholic 19, smoking alcoholic 22).	Nonsmoking light drinker: 44 (8.2)/28–68. Nonsmoking alcoholic: 52.1 (9.4)/28–68. Smoking alcoholic: 47.8 (9.2)/28–68.	Study type: Cross-sectional study. fMRI type: resting-state. Statistical method: generalized linear model.	After 5 weeks of abstinence, perfusion of frontal and parietal gray matter in nonsmoking alcoholics was significantly higher than that at baseline. The total number of cigarettes smoked per day was negatively correlated with frontal gray matter perfusion.	Strong	Neurodegeneration

(*Continued*)

Table 2
Individual studies reporting on the relationship between lifestyle factors and neurodegeneration in midlife as expressed on fMRI (*Continued*)

Study	Lifestyle factor	N	Mean age (SD)/Range	Methodology/ design	Findings	Quality assessment rating	Neurodegenerative or neuroprotective effect
Murray et al. [35]	Substance misuse	77 (alcohol-dependent 26, polysubstance use 20, light or nondrinkers 31).	Alcohol dependent: 54 (10)/NA. Polysubstance use: 45 (9)/NA. Light or nondrinkers: 47 (11)/NA.	Study type: cross-sectional study. fMRI type: resting-state. Statistical method: analyses of covariance.	Regional perfusion was significantly lower in alcoholics compared with light or nondrinkers. Greater smoking severity correlated with lower perfusion in alcohol-dependent individuals.	Strong	Neurodegeneration
Rogers et al. [36]	Alcohol	20 (alcoholic 10/healthy controls 10).	Alcoholic: 43 (12)/18–70. Control: 40 (13)/18–70.	Study type: cross-sectional study. fMRI type: task-based. Statistical method: two-sample *t*-test.	Recently abstinent alcoholic patients showed deficits in functional connectivity and recruitment of additional brain regions for the performance of a simple finger-tapping task.	Weak	Neurodegeneration
Sullivan et al. [37]	Alcohol	24 (alcoholics 12/control subjects 12).	Alcoholics: 45.7 (4.4)/38–54. Control subjects: 46.3 (5.2)/38–54.	Study type: cross-sectional study. fMRI type: task-based. Statistical method: ANOVA.	Alcoholics showed selective differences from control subjects in the CBF pattern in the anterior precuneus and CBF level in the insula, a hub of the salience network.	Moderate	Neurodegeneration
Wang et al. [38]	Substance misuse	39 (cocaine addict 20/healthy controls 19).	Cocaine addict: 42.15 (4.3)/NA. Healthy controls: 39.9 (4.5)/NA.	Study type: cross-sectional study. fMRI type: resting-state. Statistical method: two-sample *t*-test and simple regression.	Compared with controls, cocaine-addicted participants showed hypoperfusion and reduced irregularity of resting-state activity in multiple brain regions.	Moderate	Neurodegeneration
Weiland et al. [39]	Alcohol	470 (problematic alcohol use 383/controls 87).	Problematic alcohol use: 31.1 (9.3)/21–56. Controls: 25.8 (8.3)/21–53.3.	Study type: cross-sectional study. fMRI type: resting-state. Statistical method: multivariate analysis of variance.	Individuals with problematic alcohol use had significantly lower network connectivity strength than controls in the left executive control network, basal ganglia, and primary visual networks.	Weak	Neurodegeneration
Xu et al. [40]	Physical training	59 healthy adults.	66.68 (9.63)/NA.	Study type: cross-sectional study. fMRI type: resting-state. Statistical method: general linear model.	Women who engaged in strength training at least once per week exhibited significantly greater cerebrovascular perfusion than women who did not.	Strong	Neuroprotection

Abbreviations: ALFF, amplitude of low-frequency fluctuation; ANOVA, analysis of the variance; BOLD, blood oxygenation level dependent; CBF, cerebral blood flow; MANOVA, multivariate analyses of variance; NA, not available; PPS, post signal slowing; PSSI, power spectrum scale invariance; ReHo, regional homogeneity; rsFC, resting-state Functional Connectivity; SD, standard deviation; fMRI, functional magnetic resonance imaging; NFL, National Football League.

in brain health, pre-, mid-, and post-training in 37 adults who received 12-week strategy-based cognitive training versus a control group. They found increases in global and regional CBF, particularly in the default mode network (DMN) and the central executive network. In addition, they found greater connectivity in these same networks. In a follow-up, randomized control trial comparing the effects of two training protocols, cognitive training and physical training, Chapman et al. [17] describe preliminary evidence that increased cognitive and physical activities improve brain health in distinct ways. Cognitive reasoning training-enhanced frontal networks are shown to be integral to top-down cognitive control and brain resilience. In a controlled clinical trial, Hotting et al. [22] compared effects of cognitive training (spatial vs. perceptual training) and physical training (endurance training vs. nonendurance training) on spatial learning in 33 adults. Spatial learning was assessed with a virtual maze task, and at the same time, neural correlates were measured with fMRI. Only spatial training improved performance in the maze task. This improvement was accompanied by a decrease in frontal and temporal lobe activity.

3.3. Excessive internet use

Kim et al. [27] assessed the resting-state brain of individuals who were diagnosed with internet gaming disorder (IGD), as defined by Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition [42], scored over 70 on Young's Internet Addiction Test [43], and who spent more than 4 hours/day and 30 hours per week using the internet. These individuals were compared with patients with alcohol use disorder and healthy controls. Using ReHo measures, Kim et al. [27] found increased ReHo in the PCC of the IGD and alcohol use disorder groups, and decreased ReHo in the right superior temporal gyrus of those with IGD, compared with the alcohol use disorder and healthy controls group. Scores on internet addiction severity were positively correlated with ReHo in the medial frontal cortex, precuneus/PCC, and left inferior temporal cortex among those with IGD.

3.4. Fasting

Belaich et al. [13] assessed the effect of daytime fasting on six healthy male volunteers during Ramadan. Two task-based BOLD-fMRI scan sessions were performed, the first scan between the fifth and tenth days preceding the start of fasting and the second scan between the 25th and 28th day of the fasting month. The study demonstrated a significant difference in the activated brain area between maximal BOLD-fMRI signal before and during fasting. In addition, there was an increase in the volume of the activated brain area in all subjects during fasting.

3.5. Physical training

There were five interventional and two observational studies that looked at the effect of physical training on brain health in midlife, as expressed on fMRI. Chapman et al. [15] assessed brain-blood flow in 37 sedentary adults, in a clinical control trial of physical training versus a wait-list control group. Over 12 weeks, the physical training group received supervised aerobic exercise for three 1-hour sessions per week. The study found higher resting CBF in the anterior cingulate region in the physical training group as compared with the control group from baseline to post-training. In a follow-up randomized control study, Chapman et al. [17] compared the effects of two training protocols, cognitive training and physical training on brain function. The study indicates that increased cognitive and physical activities improve brain health in distinct ways. Aerobic exercise improved CBF flow in hippocampi of those with memory gains.

MacIntosh et al. [30] assessed 30 men with coronary artery disease, before and after a 6-month cardiac rehabilitation program consisting of aerobic and resistance training. CBF was associated with fitness level at baseline and greater fitness gains with exercise. McFadden et al. [32] assessed the effects of a 6-month exercise intervention on intrinsic activity in the DMN in 12 overweight or obese individuals. The intervention was associated with a reduction in DMN activity in the precuneus. This finding is thought to represent a normalization of DMN function secondary to exercise.

In a controlled clinical trial, Hotting et al. [22] assessed the effects of cognitive training (spatial vs. perceptual training) and physical training (endurance training vs. nonendurance training) on spatial learning and associated brain activation in 33 adults. Spatial learning was assessed with a virtual maze task and at the same time neural correlates were measured with fMRI. The two physical intervention groups did not improve performance in the maze task.

The findings of the two observational studies related to physical training focused on specific population groups. Hart el al. [20]. assessed aging former National Football League (NFL) players. They found that cognitive deficits and depression appear to be more common in ageing former NFL players than healthy controls. In addition, altered CBF patterns are concordant with brain regions associated with abnormal findings of neuropsychological testing in ageing former NFL players. It is noteworthy that of the 34 ex-NFL players included in the study, 32 reported a history of at least one concussion and 26 undertook neuroimaging; eight were claustrophobic and did not undergo an MRI scan. In a cross-sectional study of 59 adults recruited through a Rhode Island newspapers and an outpatient cardiology office, Xu et al. [40] found that women who engaged in strength training (weight lifting or calisthenics) at least once per week exhibited significantly greater cerebrovascular perfusion than women who did not.

3.6. Smoking

There were three cross-sectional studies identified in this systematic review that assessed the effect of smoking on brain health as expressed on fMRI. Durazzo et al. [18] investigated

the chronic effects of smoking on brain perfusion. Smokers showed significantly lower perfusion than nonsmokers in multiple brain regions (bilateral medial and lateral orbitofrontal cortices, bilateral inferior parietal lobules, bilateral superior temporal gyri, left posterior cingulate, right isthmus of cingulate, and right supramarginal gyrus) as assessed on fMRI. In addition, greater lifetime duration of smoking (adjusted for age) was related to lower perfusion in multiple brain regions. Gazdzinski et al. [19] assessed 1 week abstinent alcohol-dependent individuals in treatment (19 smokers and 10 nonsmokers) and 19 healthy light drinking nonsmoking control participants, to assess the concurrent effects of chronic alcohol and chronic smoking on cerebral perfusion. This study found that chronic cigarette smoking adversely affects cerebral perfusion in frontal and parietal gray matter of 1 week abstinent alcohol-dependent individuals. Mon et al. [44] evaluated cortical gray matter perfusion changes in short-term abstinent alcohol-dependent individuals in treatment and assessed the impact of chronic cigarette smoking on perfusion changes during abstinence. At 1 week of abstinence, frontal and parietal gray matter perfusion in smoking alcoholics was not significantly different from that in nonsmoking alcoholics. After 5 weeks of abstinence, perfusion of frontal and parietal gray matter in nonsmoking alcoholics was significantly higher than that at baseline. However, in smoking alcoholics, perfusion was not significantly different. Cigarette smoking appears to hinder perfusion improvement in abstinent alcohol-dependent individuals.

3.7. Substance misuse

Of the 12 cross-sectional studies assessing the relationship between substance misuse and neurodegeneration, nine of the studies focused on the effect of cocaine, one on heroin, one on methamphetamine, and one on polysubstance misuse.

The five task-based fMRI studies that looked at the effect of cocaine used many different fMRI measures to assess brain health, making it difficult to compare the research findings. Of the four resting-state fMRI studies assessing the effect of cocaine on brain health, both Hu et al. [23] and Kelly et al. [26] assessed the resting-state functional connectivity. Hu et al. [23] found that cocaine addiction is associated with disturbed resting-state functional connectivity in striatal-cortical circuits. Kelly et al. [26] observed reduced prefrontal interhemispheric resting-state functional connectivity in cocaine-dependent participants relative to control subjects. Liu et al. [29] used cerebral metabolic rate of oxygen (CMRO2) as a marker of neural activity and found significantly lower levels in cocaine-addicted subjects compared with healthy controls. In a multimodal MRI study, Wang et al. [38] identified hypoperfusion in the prefrontal cortex, anterior cingulate cortex, insula, right temporal cortex, and dorsolateral prefrontal cortex in cocaine addicts compared with controls.

Other than the nine studies focusing on cocaine and neurodegeneration, there were three more cross-sectional studies (one on heroin, one on methamphetamine, and one on polysubstance misuse) identified in the systematic review focusing on substance misuse. Jiang et al. [25] investigated amplitude low frequency fluctuate abnormalities in heroin users. Comparing healthy controls and heroin addicts, this resting-state fMRI study found differing spontaneous neural activity patterns in multiple regions, in the heroin addict group. Heroin addicts had decreased amplitude low frequency fluctuate in the bilateral dorsal anterior cingulate cortex, bilateral medial orbit frontal cortex, left dorsal lateral prefrontal cortex, left middle temporal gyrus, left inferior temporal gyrus, PCC, and left cuneus as well as increased amplitude low frequency fluctuate in the bilateral angular gyrus, bilateral precuneus, bilateral supramarginal gyrus, left post cingulate cortex and left middle frontal gyrus. Using a task-based fMRI protocol, May et al. [31] found that methamphetamine-dependent individuals exhibited altered responses to mechanoreceptive C-fiber stimulation in brain regions important for interoception. Murray et al [35] assessed brain perfusion in polysubstance users. They found that the combination of cigarette smoking and polysubstance use is strongly related to hypoperfusion in cortical and subcortical regions.

4. Discussion

4.1. Main findings

This systematic review identified seven lifestyle factors—physical training, cognitive training, fasting, substance misuse, alcohol, smoking, and excessive internet use—which had been researched, assessing their impact on neurodegeneration in midlife as expressed on fMRI.

4.2. Comparison with other literature

In late life, the impact of lifestyle factors on brain health has already been studied in multimodal nonpharmacological interventional studies such as the Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability [45]. The findings from this double-blind randomized control trial suggest that a 2-year multidomain intervention that included diet, exercise, and cognitive training could maintain or even improve cognitive functioning in an elderly population. In midlife, gathering evidence on the interplay between lifestyle and a broad range of markers of brain health is ongoing in projects such as the European Prevention of Alzheimer's Dementia Longitudinal Cohort Study [46]. The study is aiming to develop a well-phenotyped probability spectrum population for Alzheimer's dementia. More specifically, data on lifestyle factors and neurodegeneration in midlife as expressed on fMRI are currently being collected in the PREVENT Dementia study [2]. This is a prospective cohort study to identify midlife biomarkers of the late-onset Alzheimer's disease. The European

Prevention of Alzheimer's Dementia Longitudinal Cohort Study [46] and PREVENT Dementia studies [2] are yet to report on the relationship between lifestyle factors and neurodegeneration in midlife.

4.3. Strengths and limitations

This systematic review used a robust methodology, including a comprehensive search strategy with search terms tailored to each database examined in the review. In addition, all stages of article screening and quality assessment were undertaken independently by two reviewers. However, the systematic review has a number of limitations: There was a marked inconsistency in both study design and methodology of the included studies, which limits the confidence in our conclusions. Study populations with different demographics were assessed, different fMRI protocols were used by the studies (18 task-based and 11 resting-state), different statistical approaches to analyze the data and different fMRI outcome measures all added to this inconsistency. In addition, caution is necessary when interpreting fMRI outcome measures and how they relate to underlying neural activity. More studies are needed to provide a firmer understanding of situations when fMRI outcome measures and neural activity are coupled and when they dissociate [47].

4.4. Implications

This systematic review will contribute to our endeavor to gain a better understanding of what lifestyle factors could be reduced or enhanced to help optimize brain health in midlife and therein reduce an individual's risk of later life dementia. More specifically, the information retrieved by this systematic review could give guidance on the analysis of existing lifestyle and fMRI data in dementia prevention longitudinal cohort studies such as the PREVENT Dementia study [2]. In addition, it could help shape future multimodal nonpharmacological midlife dementia prevention studies.

4.5. Conclusions

From this systematic review, common themes have emerged on the effect of lifestyle on brain health in midlife as expressed on fMRI. All the cognitive training studies showed what could be considered a neuroprotective effect, whereas all the alcohol, smoking, and substance misuse studies showed an effect that could reflect neurodegeneration. Studies on physical training showed more variation in results. Most physical training studies showed a potential neuroprotective effect, and one study showed no effect. One study described a possible neurodegenerative effect associated with physical training; in this study by Hart et al. 2013 [20], the majority of the ex-NFL players assessed had a history of concussion. The evidence from studies on the effects of excessive internet use and fasting on brain health in midlife is too limited to allow any conclusions to be drawn.

Overall, this systematic review of the relationship between lifestyle factors and neurodegeneration in midlife as expressed on fMRI provides an evidence base for further lifestyle and fMRI research to build upon. Projects such as the European Prevention of Alzheimer's Dementia [46] and PREVENT Dementia [2] have the potential to do this and continue our drive toward the goal of reducing the global incidence of dementia.

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Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.trci.2018.04.001.

RESEARCH IN CONTEXT

1. Systematic review: The authors searched the following literature databases: MEDLINE, EMBASE, and PsycINFO. Articles were screened, and quality was assessed independently by two reviewers. The identified studies were then individually described and grouped by lifestyle factor.
2. Interpretation: The systematic review identified seven lifestyle factors (physical training, cognitive training, fasting, substance misuse, alcohol, smoking, and excessive internet use) associated with brain health in midlife as expressed on fMRI. Physical training and cognitive training have a possible neuroprotective effect. Substance misuse, alcohol, and smoking have a likely neurodegenerative effect.
3. Future directions: More research is needed into the possible neuroprotective effect of abstaining from smoking, alcohol and substance misuse, and engaging in physical and cognitive training on brain health in midlife. In addition, studies are needed to clarify the effects of fasting and excessive internet use on fMRI outcomes in midlife.

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Appendix B

EMBASE search strategy.

Search Line	Search Terms
1.	"lifestyle and related phenomena"/ or lifestyle/ or lifestyle modification/ or sedentary lifestyle/
2.	lifestyle*.mp.
3.	drinking behavior/
4.	addiction/ or alcoholism/ or exp drug dependence/ or tobacco dependence/
5.	exp smoking/ or "tobacco use"/
6.	cigarette smoking.ti,ab.
7.	exp health behavior/
8.	exp exercise/
9.	exp recreation/
10.	meditation/
11.	mindfulness/
12.	yoga/
13.	Tai Chi/
14.	exp diet/
15.	or/1-14
16.	nuclear magnetic resonance imaging/ or diffusion weighted imaging/ or functional magnetic resonance imaging/ or perfusion weighted imaging/
17.	neuroimaging/ or functional neuroimaging/
18.	spin labeling/
19.	tempone/
20.	arterial spin label*.tw.
21.	nuclear magnetic resonance spectroscopy/
22.	(magnetic resonance tomograph$ or magnetic resonance scan$ or magnetic resonance imag$ or magnetic resonance spectroscop$ or mr tomograph$ or mr scan$ or mr imag$ or mr spectroscop$ or fmri or mri or nmr or mrs).tw.
23.	16 or 17 or 18 or 19 or 20 or 21 or 22
24.	brain perfusion/
25.	brain blood flow/
26.	blood oxygenation/ or oxygenation/
27.	brain circulation/ or brain microcirculation/
28.	brain oxygen consumption/
29.	metabolism/
30.	brain chemistry/
31.	blood volume/
32.	(cerebral perfusion or hyperperfusion or hypoperfusion or cerebral blood flow).tw.
33.	or/24-32
34.	Animals/ not Human/
35.	("conference review" or editorial or letter or note or report or short survey or trade journal).pt.
36.	34 or 35
37.	15 and 23 and 33
38.	37 not 36

MEDLINE search strategy.

Search Line	Search Terms
1.	exp magnetic resonance imaging/ or exp Neuroimaging/
2.	spin labels/ or triacetoneamine-n-oxyl/
3.	arterial spin label*.tw.
4.	exp Magnetic Resonance Spectroscopy/
5.	(magnetic resonance tomograph$ or magnetic resonance scan$ or magnetic resonance imag$ or magnetic resonance spectroscop$).ti,ab.
6.	(mr tomograph$ or mr scan$ or mr imag$ or mr spectroscop$).ti,ab.
7.	(fmri or mri or nmr or mrs).ti,ab.
8.	or/1-7
9.	(brain perfusion or cerebral perfusion or hyperperfusion or hypoperfusion or oxygenation or cerebral blood flow).mp.
10.	cerebrovascular circulation/ or neurovascular coupling/
11.	metabolic phenomena/ or brain chemistry/ or metabolism/ or oxygen consumption/
12.	Brain/me [Metabolism]
13.	Blood Volume/
14.	or/9-13
15.	exp Life Style/
16.	lifestyle*.mp.
17.	exp Alcohol Drinking/
18.	exp Substance-Related Disorders/
19.	"tobacco use"/ or smoking/
20.	Health Behavior/
21.	exp Exercise/
22.	exp Leisure Activities/
23.	meditation/ or tai ji/ or yoga/ or Mindfulness/
24.	exp Diet/
25.	or/15-24
26.	Comment/ or Letter/ or Editorial/ or Autobiography/ or Biography/ or Bibliography/ or Dictionary/ or Directory/ or Interactive Tutorial/ or Lectures/
27.	Animals/ not Humans/
28.	26 or 27
29.	8 and 14 and 25
30.	29 not 28

PsycINFO search strategy.

Search Line	Search Terms
1.	exp neuroimaging/
2.	neuroimag*.ti,ab,id.
3.	(arterial spin label* or magnetic resonance tomograph$ or magnetic resonance scan$ or magnetic resonance imag$ or magnetic resonance spectroscop$ or mr tomograph$ or mr scan$ or mr imag$ or mr spectroscop$ or fmri or mri or nmr or mrs).ti,ab,id.
4.	"Brain Imaging".md.
5.	or/1-4
6.	exp lifestyle/
7.	(lifestyle* or life style*).ti,ab,id.
8.	addiction/ or exp alcoholism/ or exp drug addiction/
9.	exp drug usage/
10.	exp tobacco smoking/
11.	Health Behavior/
12.	exp exercise/ or physical fitness/ or physical activity/
13.	exp recreation/
14.	meditation/ or mindfulness/ or tai chi.ti,ab,id.
15.	diets/
16.	or/6-15
17.	cerebral blood flow/
18.	exp neurochemistry/
19.	metabolism/
20.	blood volume/ or blood circulation/ or exp blood flow/
21.	oxygenation/
22.	(brain perfusion or cerebral perfusion or hyperperfusion or hypoperfusion or cerebral blood flow or oxygenation or brain metabolism).ti,ab,id.
23.	or/17-22
24.	(animal not human).po.
25,	5 and 16 and 23
26.	25 not 24

Appendix C

UNIVERSITY of York
Centre for Reviews and Dissemination

NHS
National Institute for
Health Research

PROSPERO International prospective register of systematic reviews

The relationship between lifestyle factors and neurodegeneration in midlife as expressed on fMRI: a systematic review

Hinesh Topiwala, Graciela Muniz Terrera, Kathryn Saunderson, Tom Russ, Marshall Dozier, Craig Ritchie

Citation
Hinesh Topiwala, Graciela Muniz Terrera, Kathryn Saunderson, Tom Russ, Marshall Dozier, Craig Ritchie. The relationship between lifestyle factors and neurodegeneration in midlife as expressed on fMRI: a systematic review. PROSPERO 2016:CRD42016045237 Available from
http://www.crd.york.ac.uk/PROSPERO_REBRANDING/display_record.asp?ID=CRD42016045237

Review question(s)
What is the relationship between lifestyle factors and neurodegeneration as expressed on fMRI in midlife?

Searches
We will search the following literature databases: MEDLINE, EMBASE, and PsycINFO (all via OVID platform).

Each database will be searched using sensitive searches combining subject headings and free text terms adapted for each database. The search terms will cover the areas of neuroimaging, lifestyle, and regional changes in cerebral metabolism or blood flow, blood volume, or oxygenation.

Animal-only studies will be excluded. There are no limits by language or publication date.

Link to search strategy
http://www.crd.york.ac.uk/PROSPEROFILES/45237_STRATEGY_20160704.pdf

Types of study to be included
We will include original research articles reporting observational or interventional studies focussing on participants in midlife at the time of analysis.

Condition or domain being studied
The conditions being studied are neurodegeneration and neuroprotection. Neurodegeneration is any pathological condition primarily affecting neurons (Przedborski et al. 2003). Neuroprotection is an effect that may result in salvage, recovery or regeneration of the nervous system, its cells, structure and function (Vajda 2002).

References:

Przedborski, Serge, Miquel Vila, and Vernice Jackson-Lewis. "Series Introduction: Neurodegeneration: What is it and where are we?." The Journal of clinical investigation 111.1 (2003): 3-10.

Vajda, F. J. E. (2004). Neuroprotection and neurodegenerative disease. In Alzheimer's Disease (pp. 235-243). Humana Press.

Participants/ population
Adults in midlife (age 40 – 59).

Intervention(s), exposure(s)
The exposure in this review will be lifestyle factors. " Lifestyle is a way of living based on identifiable patterns of behaviour which are determined by the interplay between an individual's personal characteristics, social interactions, and socioeconomic and environmental living conditions" (Nutbeam, 1998).

Reference:

Nutbeam, D. (1998). Health promotion glossary. Health Promotion International, 13(4), 349-364. doi: 10.1093/heapro/13.4.349

Comparator(s)/ control
Not applicable.

Context
We will not exclude any racial, ethnic, or cultural groups and will include studies from all countries. We will include general population studies as well as those conducted in a general medical setting.

Outcome(s)
Primary outcomes
Functional magnetic resonance imaging (fMRI) is a brain imaging technique to capture regional changes in cerebral metabolism or in blood flow, volume or oxygenation in response to task activation or during rest (Noll 2001).

fMRI will be used to assess the hemodynamic correlates of neurodegeneration and neuroprotection. We will include studies using resting-state and/or task-based fMRI experimental protocols.

References:

Noll DC. A primer on MRI and functional MRI; 2001. PDF on-line: http://www.eecs.umich.edu/ dnoll/primer.pdf.

Secondary outcomes
None.

Data extraction, (selection and coding)
Titles and abstracts will be screened independently by Hinesh Topiwala (HT) and Kathryn Saunderson (KS) against the inclusion/exclusion criteria with any disagreements in the final lists of included studies resolved by discussion.

In the next stage, the full articles will be examined against the inclusion/exclusion criteria. Differences between reviewers' selections will be resolved by discussion and if required, screening by a third reviewer, Craig Ritchie (CR).

Inclusion Criteria

i. Original human research study.

ii. Population includes a lifestyle factor in midlife.

iii. Study includes a fMRI outcome in midlife.

Exclusion Criteria

i. Animal studies.

ii. Study population does not include individuals in midlife.

iii. We will exclude studies where the baseline population has a diagnosis of dementia either in general or based on specific subtypes classified using standard diagnostic criteria, e.g. the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ARDRA) (McKhann et al., 1984).

Reference:

McKhann, G., Drachman, D., Folstein, M., Katzman, R., Price, D., & Stadlan, E. M. (1984). Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and

Human Services Task Force on Alzheimer's Disease. Neurology, 34(7), 939-944.

Risk of bias (quality) assessment
The quality of randomised and non-randomised studies will be assessed with the Effective Public Health Practice Project Quality Assessment Tool (EPHPP) (Thomas, Ciliska, Dobbins, & Micucci, 2004). This tool was developed by the Effective Public Health Practice Project, Canada and has been judged suitable to be used in systematic reviews of effectiveness (Deeks et al., 2003).

References:

Deeks, J. J., Dinnes, J., D'Amico, R., Sowden, A. J., Sakarovitch, C., Song, F., . . Altman, D. G. (2003). Evaluating non-randomised intervention studies. Health Technol Assess, 7(27), iii-x, 1-173.

Thomas, B. H., Ciliska, D., Dobbins, M., & Micucci, S. (2004). A process for systematically reviewing the literature: providing the research evidence for public health nursing interventions. Worldviews Evid Based Nurs, 1(3), 176-184. doi: 10.1111/j.1524-475X.2004.04006.x

Strategy for data synthesis
The studies will be individually described and where possible, combined quantitatively in a meta-analysis.

Analysis of subgroups or subsets
Subgroup analysis will not be undertaken.

Dissemination plans
In addition to publication in a high impact, peer-reviewed journal, the findings of the systematic review will be presented locally and submitted for presentation at national and international conferences.

Contact details for further information
Dr Topiwala

3rd Floor, Kennedy Tower,

Royal Edinburgh Hospital,

Morningside Park,

Edinburgh,

EH10 5HF.

hinesh.topiwala@ed.ac.uk

Organisational affiliation of the review
Centre for Dementia Prevention, University of Edinburgh.

http://centrefordementiaprevention.com

Review team
Dr Hinesh Topiwala, Centre for Dementia Prevention, University of Edinburgh, UK.
Dr Graciela Muniz Terrera, Centre for Dementia Prevention, University of Edinburgh, UK.
Dr Kathryn Saunderson, Centre for Dementia Prevention, University of Edinburgh, UK.
Dr Tom Russ, Centre for Dementia Prevention, University of Edinburgh, UK.
Ms Marshall Dozier, Information Services, University of Edinburgh, UK.
Professor Craig Ritchie, Centre for Dementia Prevention, University of Edinburgh, UK.

Anticipated or actual start date

UNIVERSITY of York
Centre for Reviews and Dissemination

NHS
National Institute for Health Research

08 August 2016

Anticipated completion date
07 July 2017

Funding sources/sponsors
Centre for Dementia Prevention, University of Edinburgh, UK.

Conflicts of interest
None known

Language
English

Country
Scotland

Subject index terms status
Subject indexing assigned by CRD

Subject index terms
Humans; Life Style; Magnetic Resonance Imaging

Reference and/or URL for protocol
http://www.crd.york.ac.uk/PROSPEROFILES/45237_PROTOCOL_20160704.pdf

Stage of review
Ongoing

Date of registration in PROSPERO
05 August 2016

Date of publication of this revision
03 July 2017

Stage of review at time of this submission	Started	Completed
Preliminary searches	Yes	Yes
Piloting of the study selection process	Yes	Yes
Formal screening of search results against eligibility criteria	Yes	Yes
Data extraction	Yes	Yes
Risk of bias (quality) assessment	Yes	Yes
Data analysis	Yes	No

Appendix D

Individual studies excluded at full text screening.

Insufficient information
Allen et al. (208), Bowman et al. (209), Wagner et al. (210), Boots et al. (211),
Conference abstracts
Adinoff et al. (212), Alfini et al. (213), Boileau et al. (214), Bosak et al. (215), Breitner et al. (216), Bronzwaer et al. (217), Carhart-Harris. (218), Cheah et al. (219), Cheah et al. (220), Cheah et al. (221), Chen et al. (222), Childress et al. (223), Childress et al. (224), DeMarco et al. (225), Denier et al. (226), Elbejjani et al. (227), Franklin. (228), Gu et al. (229), Jagannathan et al. (230), Janse Van Rensburg et al. (231), Kroemer et al. (232), Leddy et al. (233), Lee et al. (234), Legget et al. (235), Mon et al. (236), Murray et al. (237), Nickerson et al. (238), Padula et al. (239), Rickenbacher et al. (240), Robertson et al. (241), Robertson et al. (242), Smith et al. (243), Sneider et al. (244), Stein et al. (245), Swardfager et al. (246), Tang et al. (247), Van Der Kleij (248), Weafer et al. (249) and Young et al. (250).
Not an original human research study
Abbott. (251), Anonymous. (252), Anonymous. (253), Asmaro et al. (254), Bhagavati et al. (255), Bhattacharyya et al. (256), Bogousslavsky et al. (257), Caldemeyer et al. (258), Calhoun. (259), Estruch et al. (260), Gatley et al. (261), Gottschalk et al. (262), Hurley et al. (263) and Lim et al. (264).

Study population does not include a lifestyle factor in midlife
Benwell et al. (265), Berkovich-Ohana et al. (266), Binder et al. (267), Boraxbekk et al. (268), Bray et al. (269), Chen et al. (270), Fang et al. (271), Farrell et al. (272), Gauthier et al. (273), Huang et al. (274), Jiang et al. (275), Jin et al. (276), Ludman et al. (277), Robertson et al. (278) and St-Onge et al. (279).
Study population does not include individuals in midlife
Bauernfeind et al. (280), Becker et al. (281), Berman et al. (282), Bothe et al. (283), Boujraf et al. (284), Burdette et al. (285), Buschkuehl et al. (286), Calhoun et al. (287), Chambers et al. (288), Chang et al. (289), Chang et al. (290), Chan et al. (291), Charboneau et al. (292), Ely et al. (293), Esposito et al. (294), Frank et al. (295), Fusar-Poli et al. (296), Han et al. (297), Hu et al. (298), Jager et al. (299), Kang et al. (300), Kim et al. (301), Koeneke et al. (302), Lawrence et al. (303), Leddy et al. (304), Lee et al. (305), Liu et al. (306), Lou et al. (307), Luijten et al. (308), Luijten et al. (309), Marinkovic et al. (310), Marinkovic et al. (311), Oberlin et al. (312), Olsson et al. (313), Paulus et al. (314), Pelchat et al. (315), Raj et al. (316), Rickenbacher et al. (317), Roberts et al. (318), Salomon et al. (319), Schouw et al. (320), Snel et al. (321), Tang et al. (322), Tolentino et al. (323), Valdes et al. (324), Van Hell (325), Vingerhoets et al. (326), Weafer et al. (327), Wilson et al. (328), Yalachkov et al. (329), Zeidan et al. (330).

Study does not include an fMRI outcome in midlife
Adinoff et al. (331), Breteler. (332), Domino et al. (333), Edward Roberts et al. (334), Ernst et al. (335), Fink et al. (336), Newberg et al. (337), Olin et al. (338), Voytek et al. (339), Yamashita et al. (340),
The fMRI outcome is not a proxy for neurodegeneration or neuroprotection
Addicott et al. (341), Adinoff et al. (342), Asensio et al. (343), Asensio et al. (80), Bjork et al. (344), Camchong et al. (345), Claus et al. (346), Falcone et al. (347), Froeliger et al. (348), George et al. (349), Goldstein et al. (350), Goudriaan et al. (351), Gowin et al. (352), Hu et al. (353), Hyatt et al. (354), Khushu et al. (355), Laurienti et al. (356), Lee et al. (357), Loughead et al. (358), Maas et al. (359), McHugh et al. (360), Monti et al. (285), Park et al. (361), Sinha et al. (362), Sjoerds et al. (363), Wang et al. (364), Xie et al. (365), Yamamoto et al. (366), Yang et al. (367) and Zijlstra et al. (368).

The aim of the study is not to look at the effect of a lifestyle factor on an fMRI outcome
Ances et al. (369), Archibald et al. (370), Ashare et al. (371), Blum et al. (79), Caldwell et al. (372), Carroll et al. (373), Courtney et al. (374), Denier et al. (375), Denier et al. (376), Durazzo et al. (377), Elman et al. (378), Franklin et al. (379), Franklin et al. (380), Franklin et al. (381), Franklin et al. (382), Friedman et al. (383), Hahn et al. (384), Hermann et al. (110), Herremans et al. (385), Janes et al. (386), Karch et al. (387), Karoly et al. (388), Kiefer et al. (389), Kobiella et al. (390), Kozink et al. (391), Kufahl et al. (392), Kwako et al. (393), Lam et al. (394), Langleben et al. (395), Langosch et al. (396), Loughead et al. (397), Loughead et al. (398), Lutz et al. (399), McClernon et al. (400), Meunier et al. (401), Moeller et al. (402), Myrick et al. (403), Myrick et al. (404), Risinger et al. (405), Salloum et al. (406), Schacht et al. (407), Schmidt et al. (408), Schmidt et al. (115), Sell et al. (409), Suh et al. (410), Tarumi et al. (411), Tomasi et al. (412), Van Laar et al. (413), Vatsalya et al. (414), Versace et al. (415), Wang et al. (416), Wang et al. (417), Wang et al. (418), Wang et al. (419), Warbrick et al. (420), Wetherill et al. (421), Wetherill et al. (422), Wexler et al. (423), Wiers et al. (424), Wilcox et al. (425), Xiao et al. (426) and Zlatar et al. (427).

Appendix E

```
#Linear Regression Models

#Variables used in analysis
#Cortex CBF
`mean cortex CBF`
#Hippocampus CBF
`Hippocampus mean (average of L+R)`
#Anterior Cingulate Cortex CBF
`Anterior cingulate cortex mean CBF`
#Grey Matter
GM
#White Matter
WM
#Hippocampal volume
hip_GM_vol
#Parahippocampal volume
par_GM_vol
#PVH Fazekas score
PVH_Fazekas
#DWMH Fazekas score
DWMH_Fazekas
#Intracranial volume
ICV
#Number of years of education
nbeduc
#Number of parents with dementia
number_parents_dementia

#ASL Linear Regression Models
datos<-Combined_data_151017
print(summary(reg),digits=3)

# Unadjusted Linear Regression Models for Cortex CBF
reg<-lm(`mean cortex  CBF`~PTS1to5,datos)
summary(reg)
reg<-lm(`mean cortex  CBF`~PTS6to10,datos)
summary(reg)
reg<-lm(`mean cortex  CBF`~smoker01,datos)
summary(reg)
reg<-lm(`mean cortex  CBF`~ETOHhigh,datos)
summary(reg)
reg<-lm(`mean cortex  CBF`~ETOH0,datos)
summary(reg)
reg<-lm(`mean cortex  CBF`~druguse01,datos)
summary(reg)
```

```
# Unadjusted Linear Regression Models for Hippocampus CBF
reg<-lm(`Hippocampus mean (average of L+R)`~PTS1to5,datos)
summary(reg)
reg<-lm(`Hippocampus mean (average of L+R)`~PTS6to10,datos)
summary(reg)
reg<-lm(`Hippocampus mean (average of L+R)`~smoker01,datos)
summary(reg)
reg<-lm(`Hippocampus mean (average of L+R)`~ETOHhigh,datos)
summary(reg)
reg<-lm(`Hippocampus mean (average of L+R)`~ETOH0,datos)
summary(reg)
reg<-lm(`Hippocampus mean (average of L+R)`~druguse01,datos)
summary(reg)

# Unadjusted Linear Regression Models for Anterior Cingulate Cortex CBF
reg<-lm(`Anterior cingulate cortex mean CBF`~PTS1to5,datos)
summary(reg)
reg<-lm(`Anterior cingulate cortex mean CBF`~PTS6to10,datos)
summary(reg)
reg<-lm(`Anterior cingulate cortex mean CBF`~smoker01,datos)
summary(reg)
reg<-lm(`Anterior cingulate cortex mean CBF`~ETOHhigh,datos)
summary(reg)
reg<-lm(`Anterior cingulate cortex mean CBF`~ETOH0,datos)
summary(reg)
reg<-lm(`Anterior cingulate cortex mean CBF`~druguse01,datos)
summary(reg)

# Adjusted Linear Regression Model 1 for Cortex CBF, Hippocampus CBF,
Anterior Cingulate Cortex CBF
reg<-lm(`mean cortex
CBF`~PTS1to5+age+gender01+nbeduc+apoe4+number_parents_dementia,
datos)
summary(reg)
reg<-lm(`mean cortex
CBF`~PTS6to10+age+gender01+nbeduc+apoe4+number_parents_dementia
,datos)
summary(reg)
reg<-lm(`mean cortex
CBF`~smoker01+age+gender01+nbeduc+apoe4+number_parents_dementia
,datos)
summary(reg)
reg<-lm(`mean cortex
CBF`~ETOHhigh+age+gender01+nbeduc+apoe4+number_parents_dementi
a,datos)
summary(reg)
```

```
reg<-lm(`mean cortex
CBF`~ETOHlow+age+gender01+nbeduc+apoe4+number_parents_dementia
,datos)
summary(reg)
reg<-lm(`mean cortex
CBF`~druguse01+age+gender01+nbeduc+apoe4+number_parents_dementi
a,datos)
summary(reg)
reg<-lm(`Hippocampus mean (average of L+R)`
~PTS1to5+age+gender01+nbeduc+apoe4+number_parents_dementia,datos
)
summary(reg)
reg<-lm(`Hippocampus mean (average of L+R)`
~PTS6to10+age+gender01+nbeduc+apoe4+number_parents_dementia,dato
s)
summary(reg)
reg<-lm(`Hippocampus mean (average of L+R)`
~smoker01+age+gender01+nbeduc+apoe4+number_parents_dementia,dato
s)
summary(reg)
reg<-lm(`Anterior cingulate cortex mean
CBF`~ETOHhigh+age+gender01+nbeduc+apoe4+number_parents_dementi
a,datos)
summary(reg)
reg<-lm(`Hippocampus mean (average of L+R)`
~ETOHlow+age+gender01+nbeduc+apoe4+number_parents_dementia,dato
s)
summary(reg)
reg<-lm(`Hippocampus mean (average of L+R)`
~druguse01+age+gender01+nbeduc+apoe4+number_parents_dementia,dat
os)
summary(reg)
reg<-lm(`Anterior cingulate cortex mean
CBF`~PTS1to5+age+gender01+nbeduc+apoe4+number_parents_dementia,
datos)
summary(reg)
reg<-lm(`Anterior cingulate cortex mean
CBF`~PTS6to10+age+gender01+nbeduc+apoe4+number_parents_dementia
,datos)
summary(reg)
reg<-lm(`Anterior cingulate cortex mean
CBF`~smoker01+age+gender01+nbeduc+apoe4+number_parents_dementia
,datos)
summary(reg)
reg<-lm(`Anterior cingulate cortex mean
CBF`~ETOHhigh+age+gender01+nbeduc+apoe4+number_parents_dementi
a,datos)
summary(reg)
```

```
reg<-lm(`Anterior cingulate cortex mean
CBF`~ETOHlow+age+gender01+nbeduc+apoe4+number_parents_dementia
,datos)
summary(reg)
reg<-lm(`Anterior cingulate cortex mean
CBF`~druguse01+age+gender01+nbeduc+apoe4+number_parents_dementi
a,datos)
summary(reg)

# Adjusted Linear Regression Model 2 for Cortex CBF, Hippocampus CBF,
Anterior Cingulate Cortex CBF
reg<-lm(`mean cortex
CBF`~age+gender01+nbeduc+apoe4+number_parents_dementia+PTS1to5+
PTS6to10+smoker01+ETOHhigh+ETOHlow+druguse01,datos)
summary(reg)
reg<-lm(`Hippocampus mean (average of
L+R)`~age+gender01+nbeduc+apoe4+number_parents_dementia+PTS1to5
+PTS6to10+smoker01+ETOHhigh+ETOHlow+druguse01,datos)
summary(reg)
reg<-lm(`Anterior cingulate cortex mean
CBF`~age+gender01+nbeduc+apoe4+number_parents_dementia+PTS1to5+
PTS6to10+smoker01+ETOHhigh+ETOHlow+druguse01,datos)
summary(reg)

#sMRI Linear Regression Model
datos<-Combined_data_151017
print(summary(reg),digits=3)

# Unadjusted Linear Regression Model for Grey Matter, White Matter,
Hippocampal volume, Parahippocampal volume, PVH Fazekas score,
DWMH Fazekas score
reg<-lm(GM~PTS1to5+Total_ICV,datos)
summary(reg)
reg<-lm(GM~PTS6to10+Total_ICV,datos)
summary(reg)
reg<-lm(GM ~smoker01+Total_ICV,datos)
summary(reg)
reg<-lm(GM~ETOHhigh+Total_ICV,datos)
summary(reg)
reg<-lm(GM~ETOHlow+Total_ICV,datos)
summary(reg)
reg<-lm(GM~druguse01+Total_ICV,datos)
summary(reg)
reg<-lm(WM~PTS1to5+Total_ICV,datos)
summary(reg)
reg<-lm(WM~PTS6to10+Total_ICV,datos)
summary(reg)
reg<-lm(WM ~smoker01+Total_ICV,datos)
```

```
summary(reg)
reg<-lm(WM~ETOHhigh+Total_ICV,datos)
summary(reg)
reg<-lm(WM~ETOH0+Total_ICV,datos)
summary(reg)
reg<-lm(WM~druguse01+Total_ICV,datos)
summary(reg)
reg<-lm(hip_GM_vol~PTS1to5+Total_ICV,datos)
summary(reg)
reg<-lm(hip_GM_vol~PTS6to10+Total_ICV,datos)
summary(reg)
reg<-lm(hip_GM_vol ~smoker01+Total_ICV,datos)
summary(reg)
reg<-lm(hip_GM_vol ~ETOHhigh+Total_ICV,datos)
summary(reg)
reg<-lm(hip_GM_vol ~ETOH0+Total_ICV,datos)
summary(reg)
reg<-lm(hip_GM_vol ~druguse01+Total_ICV,datos)
summary(reg)
reg<-lm(par_GM_vol~PTS1to5+Total_ICV,datos)
summary(reg)
reg<-lm(par_GM_vol~PTS6to10+Total_ICV,datos)
summary(reg)
reg<-lm(par_GM_vol~smoker01+Total_ICV,datos)
summary(reg)
reg<-lm(par_GM_vol~ETOHhigh+Total_ICV,datos)
summary(reg)
reg<-lm(par_GM_vol~ETOH0+Total_ICV,datos)
summary(reg)
reg<-lm(par_GM_vol~druguse01+Total_ICV,datos)
summary(reg)
reg<-lm(PVH_Fazekas~PTS1to5+Total_ICV,datos)
summary(reg)
reg<-lm(PVH_Fazekas~PTS6to10+Total_ICV,datos)
summary(reg)
reg<-lm(PVH_Fazekas~smoker01+Total_ICV,datos)
summary(reg)
reg<-lm(PVH_Fazekas~ETOHhigh+Total_ICV,datos)
summary(reg)
reg<-lm(PVH_Fazekas~ETOH0+Total_ICV,datos)
summary(reg)
reg<-lm(PVH_Fazekas~druguse01+Total_ICV,datos)
summary(reg)
reg<-lm(DWMH_Fazekas~PTS1to5+Total_ICV,datos)
summary(reg)
reg<-lm(DWMH_Fazekas~PTS6to10+Total_ICV,datos)
summary(reg)
reg<-lm(DWMH_Fazekas ~smoker01+Total_ICV,datos)
```

```
summary(reg)
reg<-lm(DWMH_Fazekas~ETOHhigh+Total_ICV,datos)
summary(reg)
reg<-lm(DWMH_Fazekas~ETOH0+Total_ICV,datos)
summary(reg)
reg<-lm(DWMH_Fazekas~druguse01+Total_ICV,datos)
summary(reg)

# Adjusted Linear Regression Model 1 for Grey Matter, White Matter,
Hippocampal volume, Parahippocampal volume, PVH Fazekas score,
DWMH Fazekas score
reg<-
lm(GM~PTS1to5+age+gender01+nbeduc+apoe4+number_parents_dementi
a+Total_ICV,datos)
summary(reg)
reg<-
lm(GM~PTS6to10+age+gender01+nbeduc+apoe4+number_parents_dement
ia+Total_ICV,datos)
summary(reg)
reg<-
lm(GM~smoker01+age+gender01+nbeduc+apoe4+number_parents_dementi
a+Total_ICV,datos)
summary(reg)
reg<-
lm(GM~ETOHhigh+age+gender01+nbeduc+apoe4+number_parents_demen
tia+Total_ICV,datos)
summary(reg)
reg<-
lm(GM~ETOH0+age+gender01+nbeduc+apoe4+number_parents_dementia
+Total_ICV,datos)
summary(reg)
reg<-
lm(GM~druguse01+age+gender01+nbeduc+apoe4+number_parents_demen
tia+Total_ICV,datos)
summary(reg)
reg<-
lm(WM~PTS1to5+age+gender01+nbeduc+apoe4+number_parents_dementi
a+Total_ICV,datos)
summary(reg)
reg<-
lm(WM~PTS6to10+age+gender01+nbeduc+apoe4+number_parents_dement
ia+Total_ICV,datos)
summary(reg)
reg<-
lm(WM~smoker01+age+gender01+nbeduc+apoe4+number_parents_dement
ia+Total_ICV,datos)
summary(reg)
```

```
reg<-
lm(WM~ETOHhigh+age+gender01+nbeduc+apoe4+number_parents_demen
tia+Total_ICV,datos)
summary(reg)
reg<-
lm(WM~ETOH0+age+gender01+nbeduc+apoe4+number_parents_dementia
+Total_ICV,datos)
summary(reg)
reg<-
lm(WM~druguse01+age+gender01+nbeduc+apoe4+number_parents_demen
tia+Total_ICV,datos)
summary(reg)
reg<-lm(hip_GM_vol
~PTS1to5+age+gender01+nbeduc+apoe4+number_parents_dementia+Total
_ICV,datos)
summary(reg)
reg<-lm(hip_GM_vol
~PTS6to10+age+gender01+nbeduc+apoe4+number_parents_dementia+Tot
al_ICV,datos)
summary(reg)
reg<-lm(hip_GM_vol
~smoker01+age+gender01+nbeduc+apoe4+number_parents_dementia+Tot
al_ICV,datos)
summary(reg)
reg<-lm(hip_GM_vol
~ETOHhigh+age+gender01+nbeduc+apoe4+number_parents_dementia+Tot
al_ICV,datos)
summary(reg)
reg<-lm(hip_GM_vol
~ETOH0+age+gender01+nbeduc+apoe4+number_parents_dementia+Total_
ICV,datos)
summary(reg)
reg<-lm(hip_GM_vol
~druguse01+age+gender01+nbeduc+apoe4+number_parents_dementia+Tot
al_ICV,datos)
summary(reg)
reg<-
lm(par_GM_vol~PTS1to5+age+gender01+nbeduc+apoe4+number_parents_
dementia+Total_ICV,datos)
summary(reg)
reg<-
lm(par_GM_vol~PTS6to10+age+gender01+nbeduc+apoe4+number_parents
_dementia+Total_ICV,datos)
summary(reg)
reg<-
lm(par_GM_vol~smoker01+age+gender01+nbeduc+apoe4+number_parents
_dementia+Total_ICV,datos)
summary(reg)
```

```
reg<-
lm(par_GM_vol~ETOHhigh+age+gender01+nbeduc+apoe4+number_parents
_dementia+Total_ICV,datos)
summary(reg)
reg<-
lm(par_GM_vol~ETOH0+age+gender01+nbeduc+apoe4+number_parents_d
ementia+Total_ICV,datos)
summary(reg)
reg<-
lm(par_GM_vol~druguse01+age+gender01+nbeduc+apoe4+number_parent
s_dementia+Total_ICV,datos)
summary(reg)
reg<-
lm(PVH_Fazekas~PTS1to5+age+gender01+nbeduc+apoe4+number_parent
s_dementia+Total_ICV,datos)
summary(reg)
reg<-
lm(PVH_Fazekas~PTS6to10+age+gender01+nbeduc+apoe4+number_paren
ts_dementia+Total_ICV,datos)
summary(reg)
reg<-
lm(PVH_Fazekas~smoker01+age+gender01+nbeduc+apoe4+number_paren
ts_dementia+Total_ICV,datos)
summary(reg)
reg<-
lm(PVH_Fazekas~ETOHhigh+age+gender01+nbeduc+apoe4+number_pare
nts_dementia+Total_ICV,datos)
summary(reg)
reg<-
lm(PVH_Fazekas~ETOH0+age+gender01+nbeduc+apoe4+number_parents
_dementia+Total_ICV,datos)
summary(reg)
reg<-
lm(PVH_Fazekas~druguse01+age+gender01+nbeduc+apoe4+number_pare
nts_dementia+Total_ICV,datos)
summary(reg)
reg<-
lm(DWMH_Fazekas~PTS1to5+age+gender01+nbeduc+apoe4+number_pare
nts_dementia+Total_ICV,datos)
summary(reg)
reg<-
lm(DWMH_Fazekas~PTS6to10+age+gender01+nbeduc+apoe4+number_par
ents_dementia+Total_ICV,datos)
summary(reg)
reg<-
lm(DWMH_Fazekas~smoker01+age+gender01+nbeduc+apoe4+number_par
ents_dementia+Total_ICV,datos)
summary(reg)
```

```
reg<-
lm(DWMH_Fazekas~ETOHhigh+age+gender01+nbeduc+apoe4+number_pa
rents_dementia+Total_ICV,datos)
summary(reg)
reg<-
lm(DWMH_Fazekas~ETOH0+age+gender01+nbeduc+apoe4+number_paren
ts_dementia+Total_ICV,datos)
summary(reg)
reg<-
lm(DWMH_Fazekas~druguse01+age+gender01+nbeduc+apoe4+number_pa
rents_dementia+Total_ICV,datos)
summary(reg)

# Adjusted Linear Regression Model 2 for Grey Matter, White Matter,
Hippocampal volume, Parahippocampal volume, PVH Fazekas score,
DWMH Fazekas score
reg<-
lm(GM~age+gender01+nbeduc+apoe4+number_parents_dementia+Total_IC
V+PTS1to5+PTS6to10+smoker01+ETOHhigh+ETOH0+druguse01,datos)
summary(reg)
reg<-
lm(WM~age+gender01+nbeduc+apoe4+number_parents_dementia+Total_I
CV+PTS1to5+PTS6to10+smoker01+ETOHhigh+ETOH0+druguse01,datos)
summary(reg)
reg<-
lm(hip_GM_vol~age+gender01+nbeduc+apoe4+number_parents_dementia+
Total_ICV+PTS1to5+PTS6to10+smoker01+ETOHhigh+ETOH0+druguse01,d
atos)
summary(reg)
reg<-
lm(par_GM_vol~age+gender01+nbeduc+apoe4+number_parents_dementia+
Total_ICV+PTS1to5+PTS6to10+smoker01+ETOHhigh+ETOH0+druguse01,d
atos)
summary(reg)
reg<-
lm(PVH_Fazekas~age+gender01+nbeduc+apoe4+number_parents_dementi
a+Total_ICV+PTS1to5+PTS6to10+smoker01+ETOHhigh+ETOH0+druguse0
1,datos)
summary(reg)
reg<-
lm(DWMH_Fazekas~age+gender01+nbeduc+apoe4+number_parents_deme
ntia+Total_ICV+PTS1to5+PTS6to10+smoker01+ETOHhigh+ETOH0+drugus
e01,datos)
summary(reg)
```

Appendix F

Linear regression diagnostic plots

To draw conclusions about a midlife population based on regression analysis undertaken on the PREVENT Dementia cohort (119), several assumptions must be true (428):

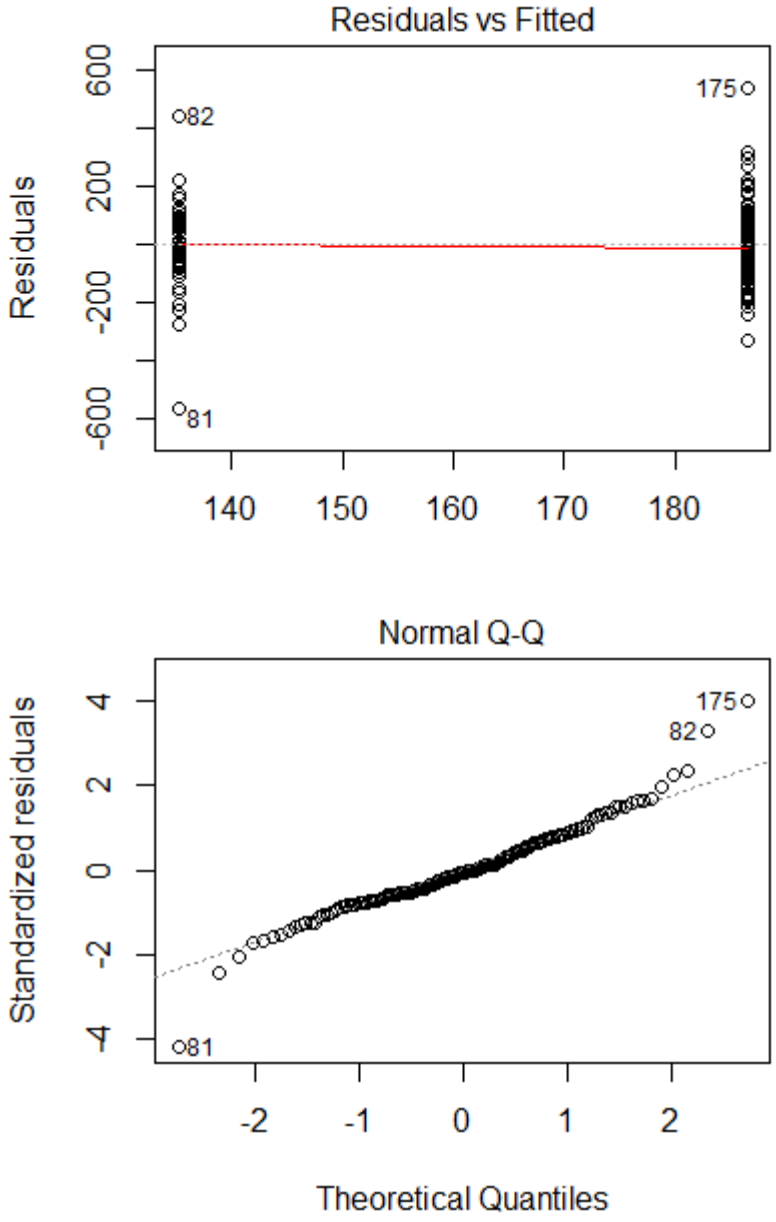
- Variable types: All predictor variables must be quantitative or categorical, with two categories. The outcome variable must be quantitative and continuous (428).
- Non-zero variance: The predictors should have some variation in value (428).
- No perfect multicollinearity: There should be no perfect linear relationship between two or more of the predictors (428).
- Predictors are uncorrelated with external variables: External variables are variables that haven't been included in the regression model which influence the outcome variable. This assumption means that there should be no external variables that correlate with any of the variables included in the regression model (428).
- Homoscedasticity: The residuals at each level of the predictors should have the same variance. The residual is the difference between the observed value of the dependent variable and the predicted value.
- Independent errors: For any two observations the residual terms should be uncorrelated (428).
- Normally distributed errors: It is assumed that the residuals in the model are random, normally distributed variables with a mean of 0.

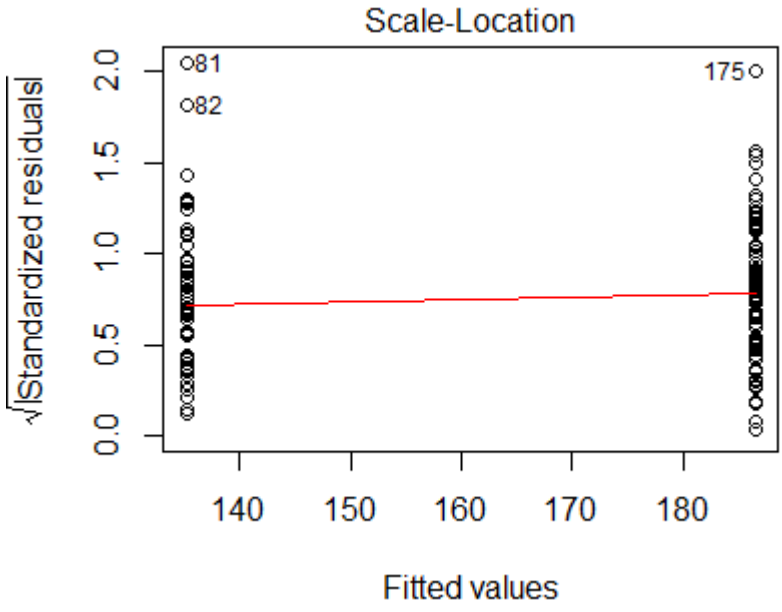
- Independence: It is assumed that all of the values of the outcome variable are independent (428).
- Linearity: It is assumed that the relationship that is being modelled is a linear one (428).

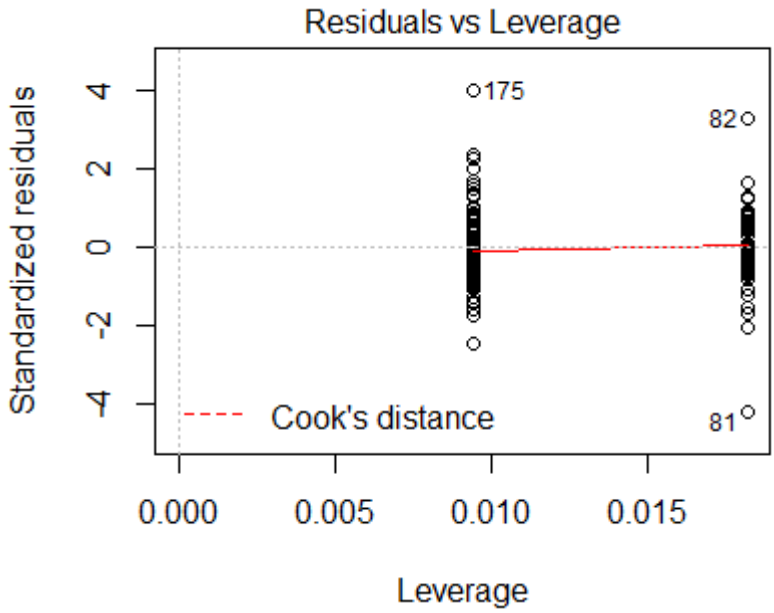
There were four diagnostic plots used to evaluate the linear regression model assumptions:

1. Residuals vs Fitted**:** If the assumptions of linearity and homoscedasticity are met, the diagnostic plot will look like a random array of dots dispersed around zero (150).
2. Normal Q-Q**:** Used to examine whether the residuals are normally distributed. The straight line in this plot represents a normal distribution, and the points represent the observed residuals. Therefore, in a perfectly normally distributed data set, all points will lie on the line (150).
3. Scale-Location: The diagnostic plot is used to check the assumption of homoscedasticity, it shows if residuals are spread equally along the ranges of predictors (429).
4. Residuals vs Leverage**:** Used to identify influential cases, that is extreme values that might influence the regression results (429). Cook's distance is shown on the diagnostic plot. Cook's distance is a measure of the overall influence of a case on the model.

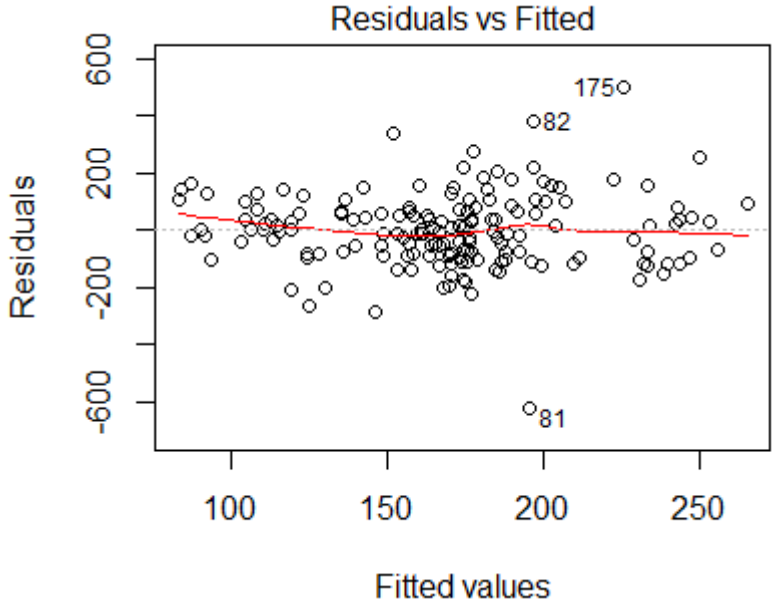
Diagnostic plots for the unadjusted linear regression model of the relationship between current and ex-substance misuse and anterior cingulate cortex cerebral blood flow in midlife as expressed on fMRI

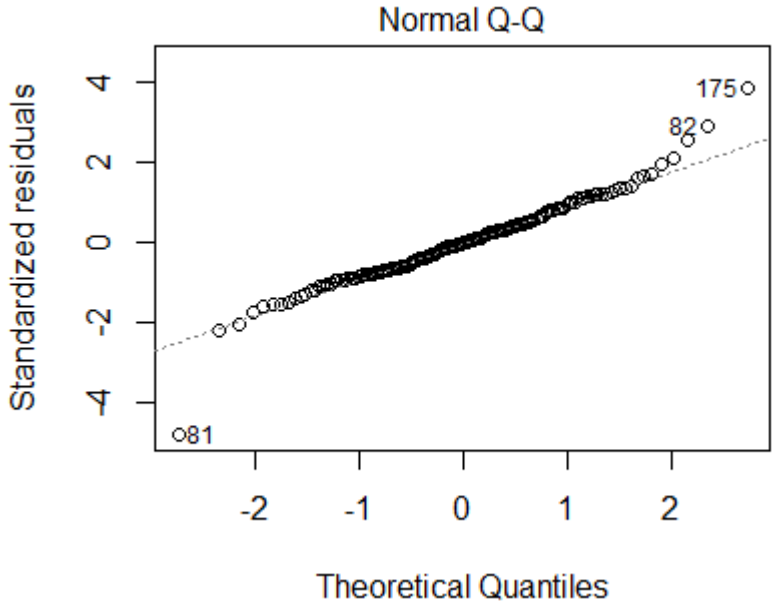


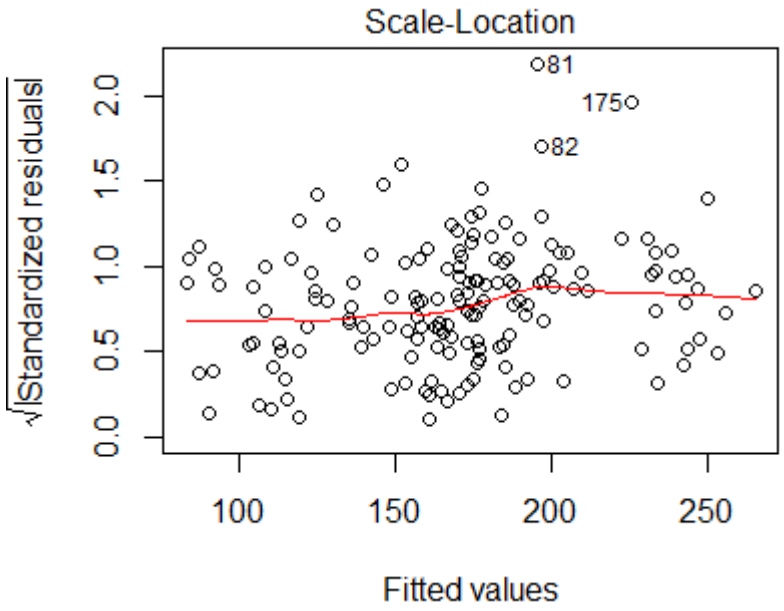

Scale-Location
√|Standardized residuals|
0.0
0.5
1.0
1.5
2.0
81
82
175
140
150
160
170
180
Fitted values

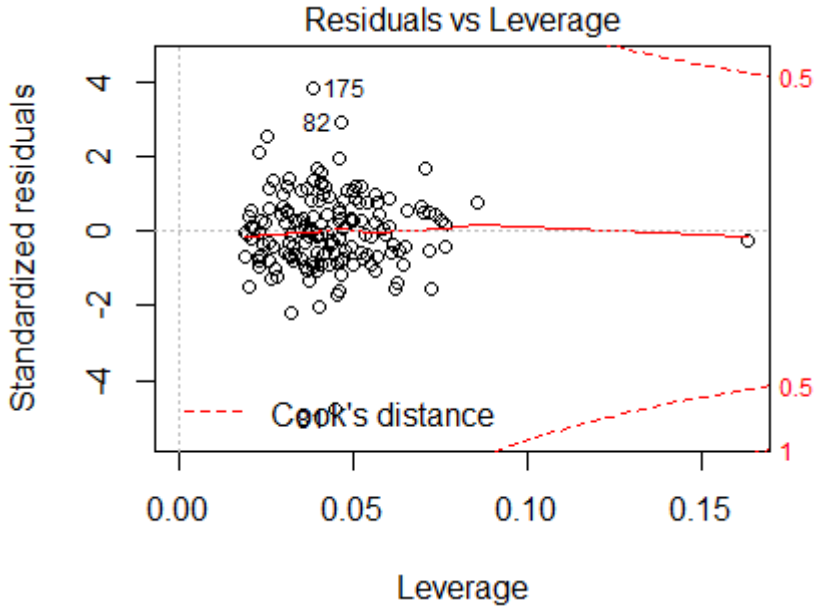

Residuals vs Leverage
Standardized residuals
-4
-2
0
2
4
175
82
81
Cook's distance
0.000
0.005
0.010
0.015
Leverage

Diagnostic plots for the adjusted multiple linear regression (model 1: age + gender + education + *APOE* ε4 allele+ number of parents with dementia) of the relationship between current and ex-substance misuse and anterior cingulate cortex cerebral blood flow in midlife as expressed on fMRI.

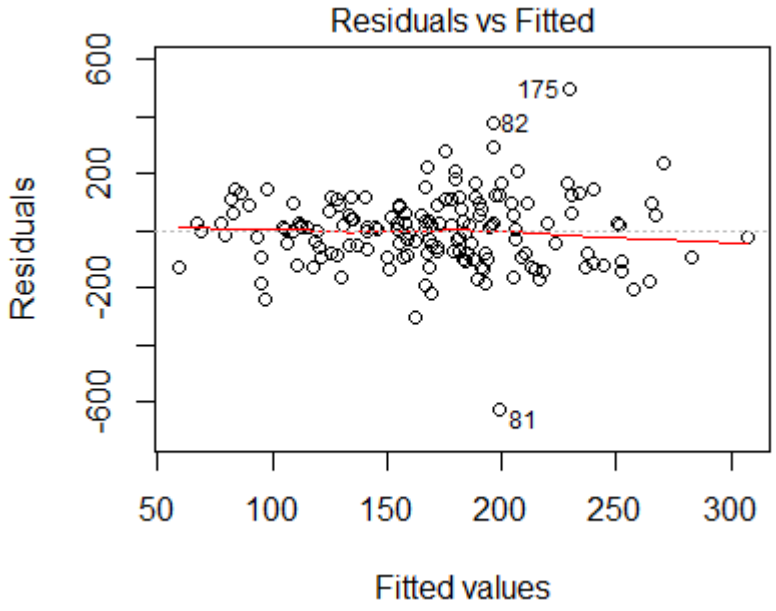


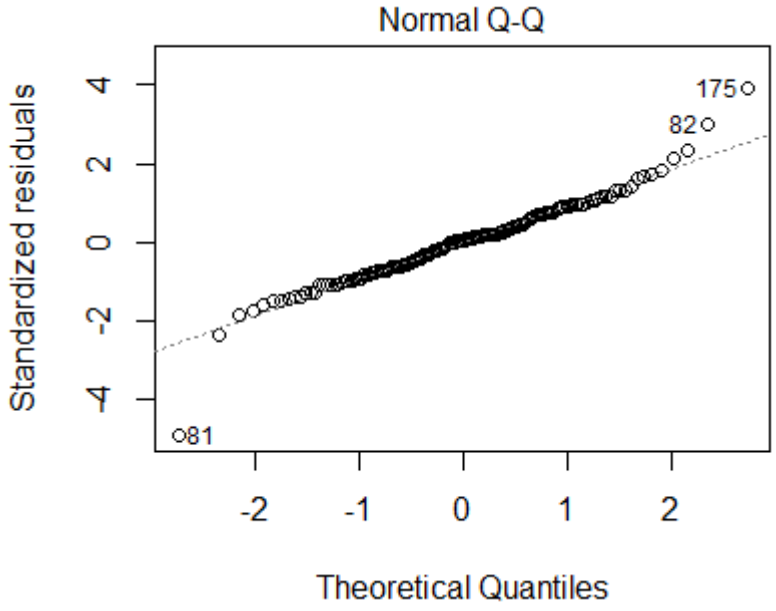


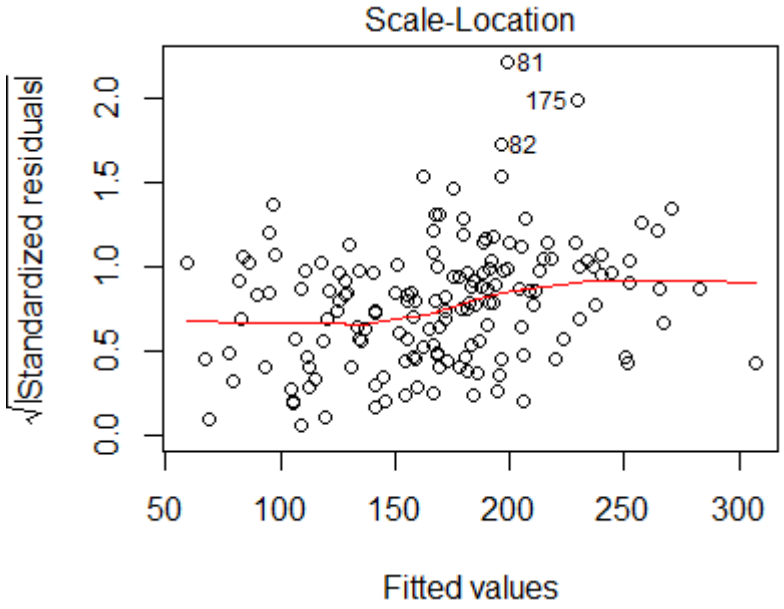

Scale-Location
√|Standardized residuals|
0.0
0.5
1.0
1.5
2.0
81
175
82
100
150
200
250
Fitted values

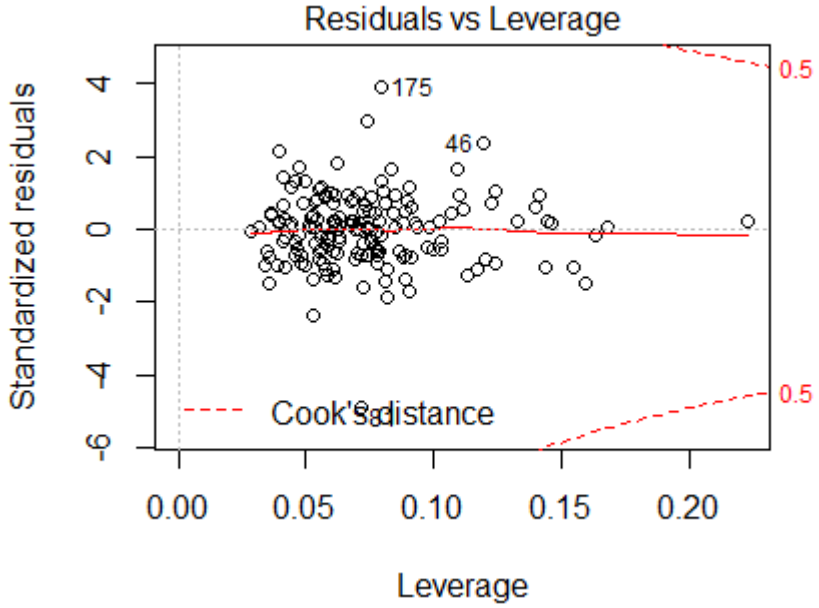

Residuals vs Leverage
Standardized residuals
-4
-2
0
2
4
175
82
Cook's distance
0.5
0.5
1
0.00
0.05
0.10
0.15
Leverage

Diagnostic plots for the adjusted linear regression (model 2: adjusted for age + gender + education + *APOE* ε4 allele + number of parents with dementia + physical training score + smoking status + alcohol drinking status + substance misuse status) of the relationship between lifestyle factors and anterior cingulate cortex cerebral blood flow in midlife as expressed on fMRI.






Scale-Location
√|Standardized residuals|
81
175
82
0.0
0.5
1.0
1.5
2.0
50
100
150
200
250
300
Fitted values


Residuals vs Leverage
Standardized residuals
175
46
0.5
0.5
Cook's distance
4
2
0
-2
-4
-6
0.00
0.05
0.10
0.15
0.20
Leverage